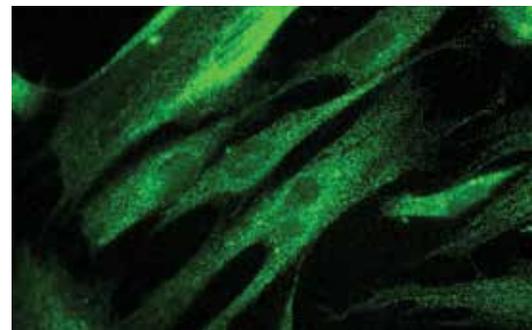
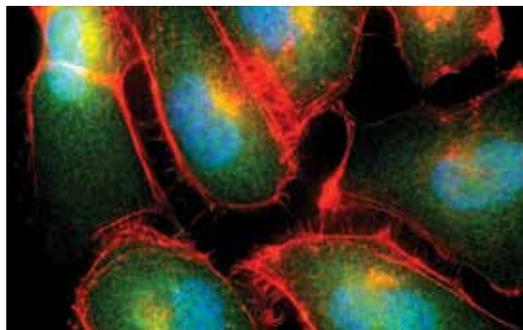
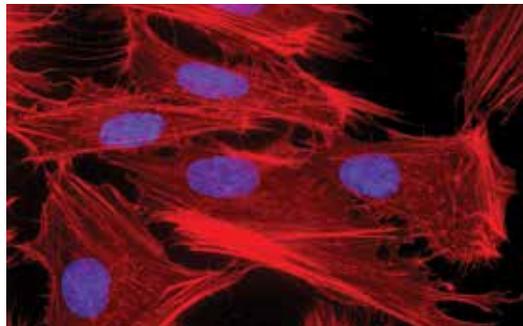


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## Genetic Alteration Panels



# GENETIC ALTERATION PANELS

## Table of Contents

AKT Genetic Alteration Panel (ATCC® No. TCP-1029™) .....	1
BRAF Genetic Alteration Panel (ATCC® No. TCP-1032™) .....	5
EGFR Genetic Alteration Panel (ATCC® No. TCP-1027™) .....	9
ERK genetic alteration cell panel (ATCC® No. TCP-1033™) .....	13
FGFR Genetic Alteration Cell Panel (ATCC® No. TCP-1034™) .....	16
MET genetic alteration cell panel (ATCC® No. TCP-1036™) .....	20
MYC genetic alteration cell panel (ATCC® No. TCP-1035™) .....	23
PI3K Genetic Alteration Panel (ATCC® No. TCP-1028™) .....	27
PTEN Genetic Alteration Panel (ATCC® No. TCP-1030™) .....	30
RAS genetic alteration cell panel (ATCC® No. TCP-1031™) .....	34

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# AKT GENETIC ALTERATION PANEL (ATCC® NO. TCP-1029™)

AKT is a serine–threonine protein kinase expressed as three isoforms — AKT1, AKT2 and AKT3. AKT activation is initiated by translocation to the plasma membrane, which is mediated by receptor tyrosine kinase-PI3K pathway. Activated AKT phosphorylates many key proteins such as glycogen synthase kinase 3 and the FOXOs, and regulates cell survival, proliferation and other cellular processes. Amplification of AKT1 and AKT2 has been discovered in various common tumor types. AKT1 is linked to tumor cell survival and growth, whereas AKT2 is linked to tumor invasiveness. The AKT genetic alteration cell panel (ATCC® No. TCP-1029™) is composed of eight selected human tumor cell lines from the common cancer types that carry various degrees of AKT gene copy number changes. The AKT1 and AKT2 gene alteration status of each cell line has been sequenced and validated by ATCC. This panel is useful for AKT pathway research, as well as for developing pan AKT inhibitors or isoform specific AKT inhibitors as anti-cancer therapeutics.

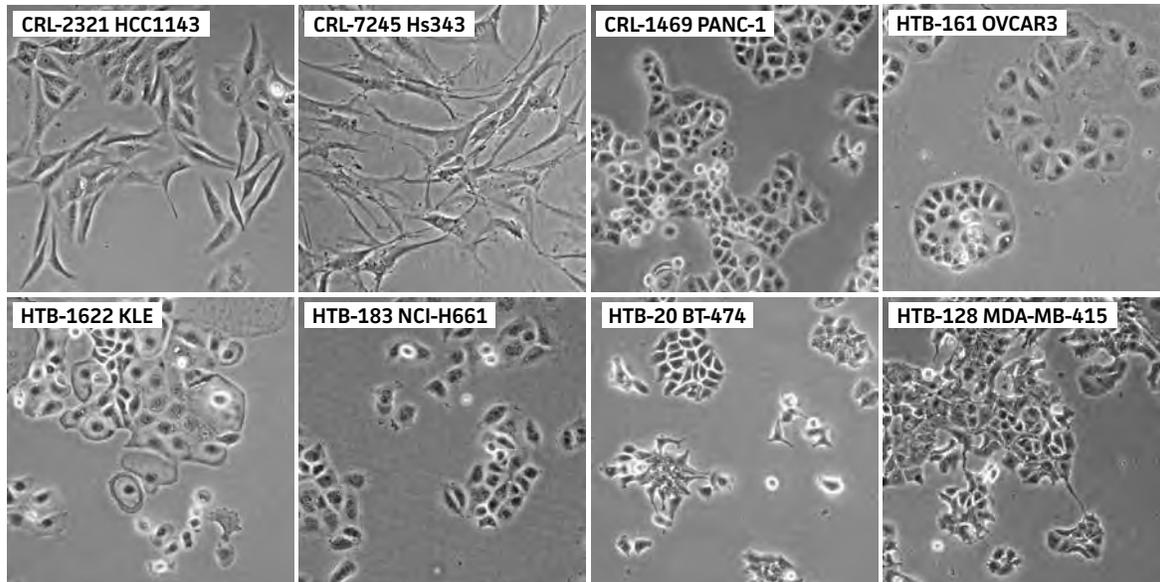
## ATCC® No. TCP-1029™ AKT Genetic Alteration Cell Panel

ATCC® No.	Name	Gene	AKT1 copy number variation	AKT2 copy number variation	phosphor-AKT level	Tumor source
CRL-2321™	HCC1143	AKT	amplification	–	–	breast
CRL-7245™	Hs 343.T	AKT	–	–	–	breast
CRL-1469™	PANC-1	AKT	–	amplification	–	pancreas
HTB-161™	NIH:OVCA3	AKT	–	amplification	–	ovary
CRL-1622™	KLE	AKT	–	amplification	–	endometrium
HTB-183™	NCI-H661	AKT	–	amplification	–	lung
HTB-20™	BT-474	AKT	–	–	high	breast
HTB-128™	MDA-MB-415	AKT	–	–	high	breast

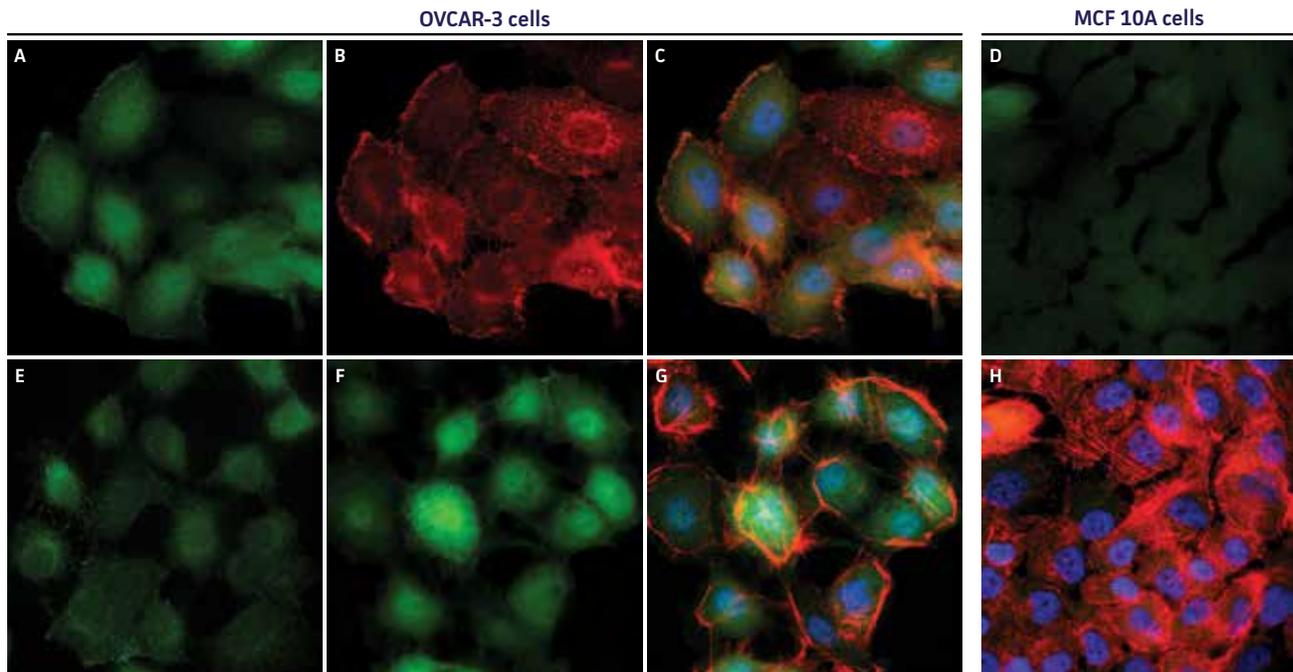
- Somatic mutations analysis was performed by illumina next generation sequencing with greater than 100x coverage. Gene copy number variation analysis was performed by using qBiomarker Copy Number PCR Assay kit from SA Biosciences.
- A list of wild type control cell lines is recommended at the end of this file.

## ATCC® No. TCP-1029™ Recommended Culture Conditions

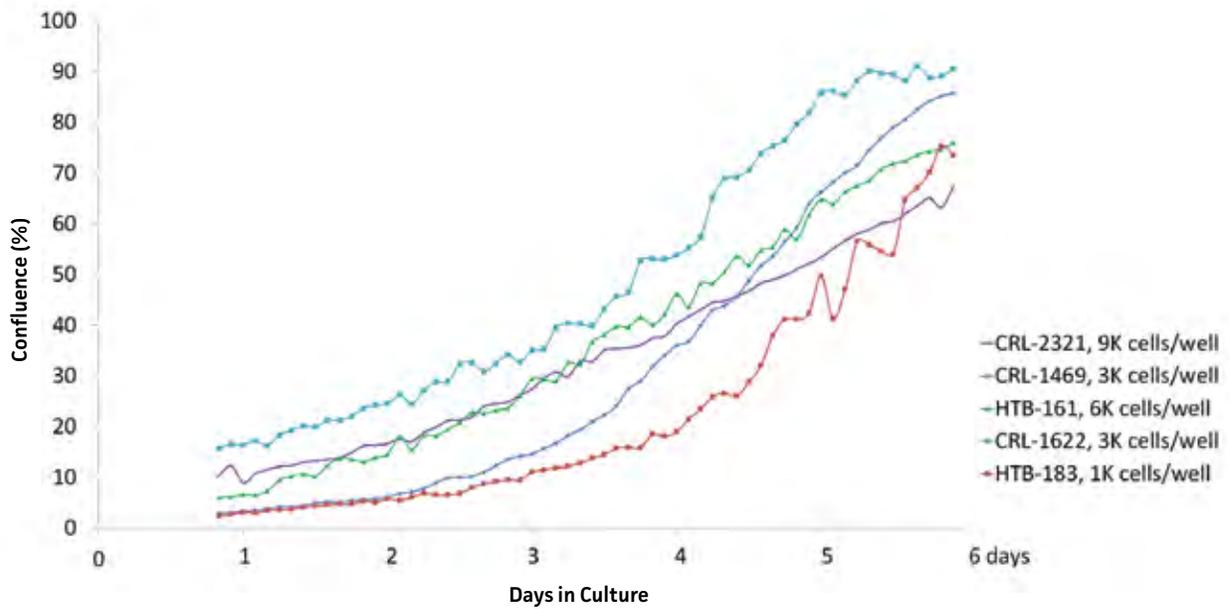
ATCC® No.	Name	Tumor source	Histology	Media	Culture recommendation
CRL-2321™	HCC1143	breast	ductal carcinoma	RPMI-1640 + 10% FBS	seeding density: 1.2 x 10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 6-7 days, 1:2 to 1:4 split
CRL-7245™	Hs 343.T	breast	adenocarcinoma	DMEM + 10% FBS	seeding density: 8.0 x 10 <sup>3</sup> cells/cm <sup>2</sup> , subculture every 6-7 days, 1:2 to 1:4 split
CRL-1469™	PANC-1	pancreas	ductal carcinoma	DMEM + 10% FBS	seeding density: 1.0 x 10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 6-7 days, 1:8 to 1:10 split
HTB-161™	NIH:OVCA3	ovary	adenocarcinoma	RPMI-1640 +10 µg/mL insulin +20% FBS	seeding density: 2.0 x 10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 5-6 days, 1:2 to 1:4 split
CRL-1622™	KLE	endometrium	adenocarcinoma	DMEM:F12 Medium + 10% FBS	seeding density: 1.5 x 10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 6-7 days, 1:5 to 1:10 split
HTB-183™	NCI-H661	lung	large cell carcinoma	RPMI-1640 + 10% FBS	seeding density: 5.0 x 10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 4-5 days, 1:6 to 1:12split
HTB-20™	BT-474	breast	ductal carcinoma	HybriCare + 10% FBS	seeding density: 3.0 x 10 <sup>3</sup> cells/cm <sup>2</sup> , subculture every 6-7 days, 1:6 to 1:9 split
HTB-128™	MDA-MB-415	breast	adenocarcinoma	Leibovitz's L-15 + 10 µg/mL insulin and 10 µg/mL glutathione + 15% FBS	seeding density: 6.0 x 10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 6-7 days, 1:2 to 1:3 split



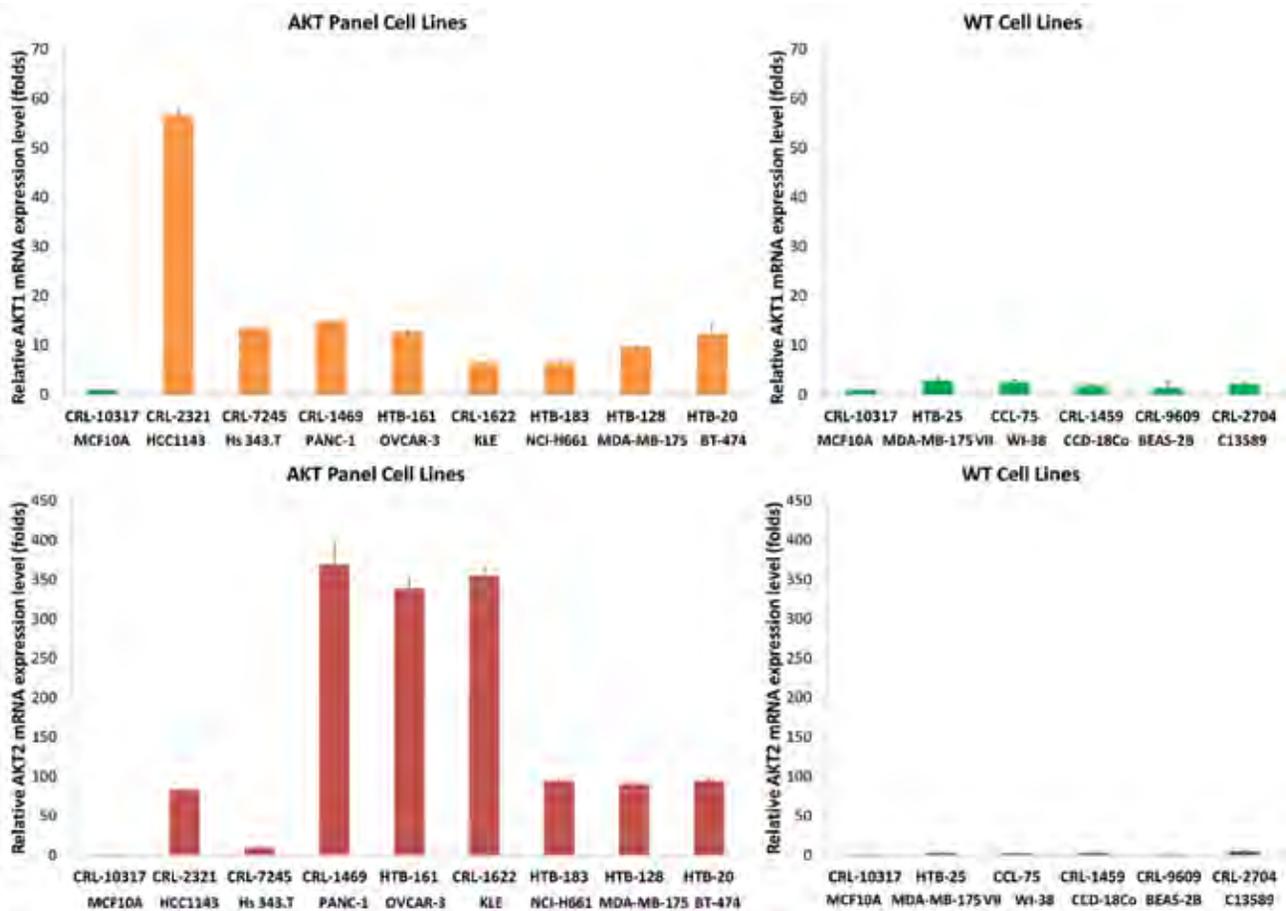
**Figure 1.** Cell morphology of eight tumor cell lines in the AKT Genetic Alteration Cell Panel. Cells were maintained in ATCC recommended culture condition. Cell morphology was observed under Nikon™ microscopy, and images of the indicated cell lines were captured by an Olympus® digital camera.



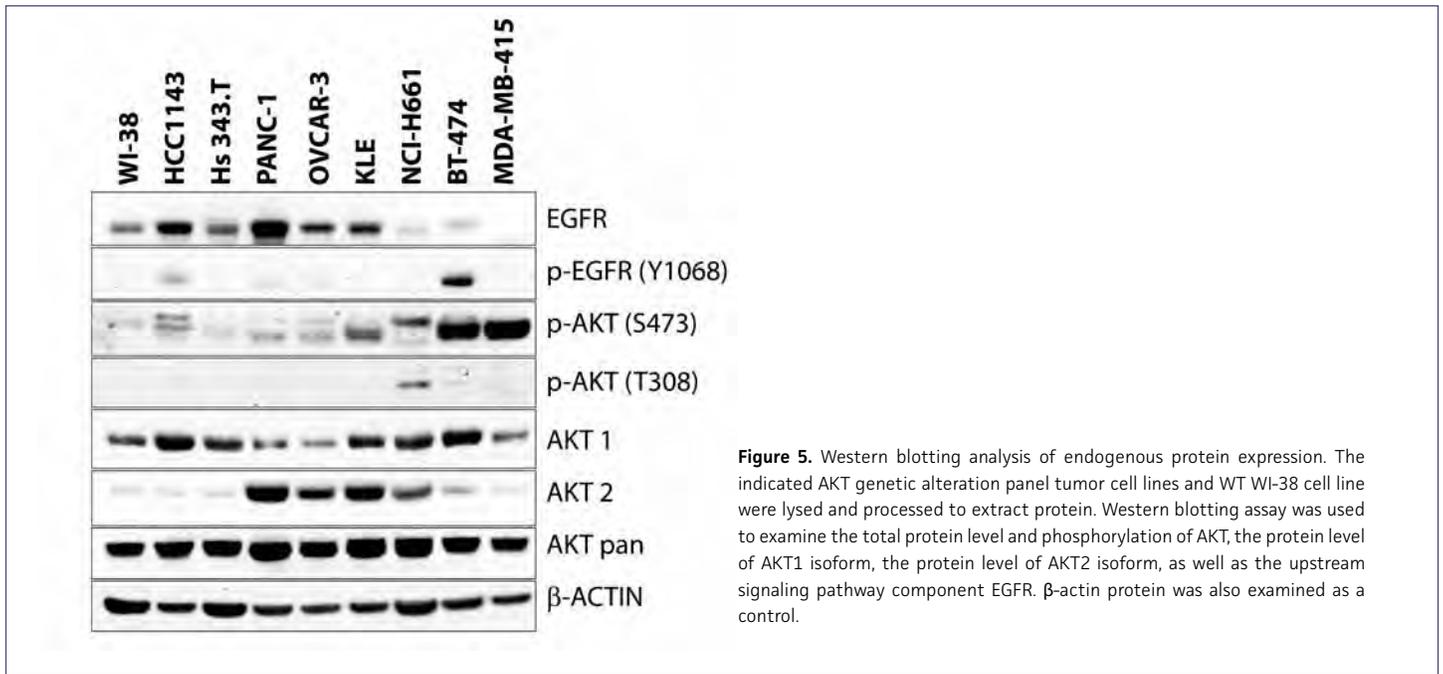
**Figure 2.** Immunofluorescence staining. The indicated AKT genetic alteration cells and recommended WT control cells were grown on collagen coated coverslips. Cells were fixed with 4% paraformaldehyde. A), AKT2 was stained with AKT2 primary antibody and Alexa Fluor 488 secondary antibody (green). B) EGFR, the upstream of AKT, was stained with EGFR primary antibody and Alexa Fluor 594 secondary antibody (red). C). Merged images of AKT2, EGFR and nuclei (blue). E), phosphor-AKT was stained with phosphor- AKT(S473) primary antibody and Alexa Fluor 488 secondary antibody (green). D) and F), pan AKT was stained with pan AKT primary antibody and Alexa Fluor 488 secondary antibody (green). G) and H) F-actin was visualized with phalloidin Alexa Fluor 594 (red). Nuclei of the cells were visualized with Hoechst 33342 (blue). Multichannel merged images of pan AKT, F-actin and nuclei were shown as three-color images.



**Figure 3.** Cell growth kinetics. The indicated genetic alteration panel tumor cell lines were cultured in ATCC recommended media, and plated in 96 well plate at 1000 cells/well - 9000 cells/well. The cell growth kinetics were constantly monitored for 6 days by using a label-free automated IncuCyte live-cell imaging system (Essen Bioscience).



**Figure 4.** Real time PCR analysis of mRNA levels. The mRNA expression level of AKT1, AKT2 and 36B4 were determined by real time quantitative PCR. Relative AKT1 mRNA expression and AKT2 mRNA expression of indicated AKT genetic alteration cells were calculated by comparing to WT MCF10A cells after normalization to the housekeeping gene 36B4. The data of recommended WT control cell lines was also provided. Tumor cell lines within the panel were shown as orange or red, and WT control cell lines were shown as green or blue.



#### Recommended Control Cell Lines

ATCC® No.	Name	Tissue Source	Cell Type	Histology
HTB-25™	MDA-MB-175-VII	breast	epithelial	ductal carcinoma
CRL-10317™	MCF 10A	breast	epithelial	normal
CCL-75™	WI-38	lung	fibroblast	normal
CRL-9609™	BEAS-2B	lung	epithelial	normal
CRL-1459™	CCD-18Co	colon	fibroblast	normal
CRL-2704™	C13589	hematopoietic and lymphoid	B lymphoblast	normal

# BRAF GENETIC ALTERATION PANEL (ATCC® NO. TCP-1032™)

The BRAF gene is a proto-oncogene encoding the BRAF protein, a serine/threonine kinase of the RAF family that acts downstream of RAS and upstream of MEK in the MAPK/ERK signaling pathway. BRAF mediates cell division, proliferation, and differentiation in response to a host of stimuli. Mutations in BRAF lead to excessive cellular proliferation and enhanced survival, and often underlie birth defects, thyroid and skin cancer. The BRAF gene is most frequently mutated at codon 600. For example, the BRAF V600E mutation has been found in more than 60% of melanomas, as well as 7 to 8% of other cancers. The BRAF Genetic Alteration Cell Panel (ATCC® No. TCP-1032™) is composed of eight selected human tumor cell lines from various common cancer types that carry BRAF hotspot mutations in codon 600. The BRAF mutation status of each cell line has been sequenced and validated by ATCC. This panel is useful for BRAF pathway research and BRAF inhibitors anti-cancer drug discovery.

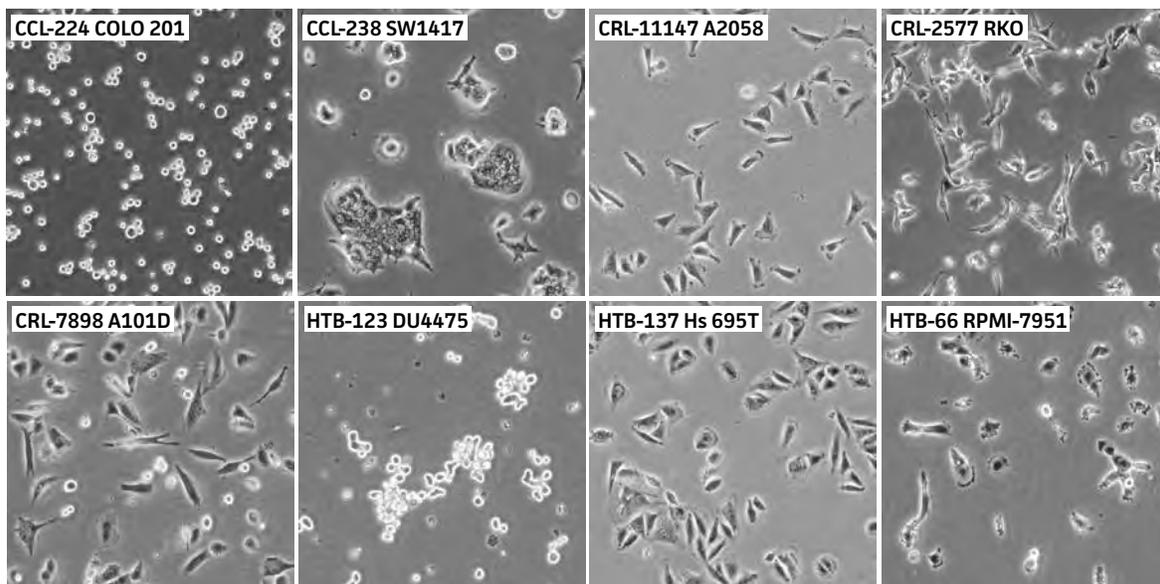
## ATCC® No. TCP-1032™ BRAF Genetic Alteration Cell Panel

ATCC® No.	Name	Gene	DNA change	Zygoty	Amino acid change	Tumor source
CCL-224™	COLO 201	BRAF	c.1799T>A	Heterozygous	p.V600E	colon
CCL-238™	SW1417	BRAF	c.1799T>A	Heterozygous	p.V600E	colon
CRL-11147™	A2058	BRAF	c.1799T>A	Heterozygous	p.V600E	skin
CRL-2577™	RKO	BRAF	c.1799T>A	Heterozygous	p.V600E	colon
CRL-7898™	A101D	BRAF	c.1799T>A	Heterozygous	p.V600E	skin
HTB-123™	DU4475	BRAF	c.1799T>A	Heterozygous	p.V600E	breast
HTB-137™	Hs 695T	BRAF	c.1799T>A	Heterozygous	p.V600E	skin
HTB-66™	RPMI-7951	BRAF	c.1799T>A	Heterozygous	p.V600E	skin

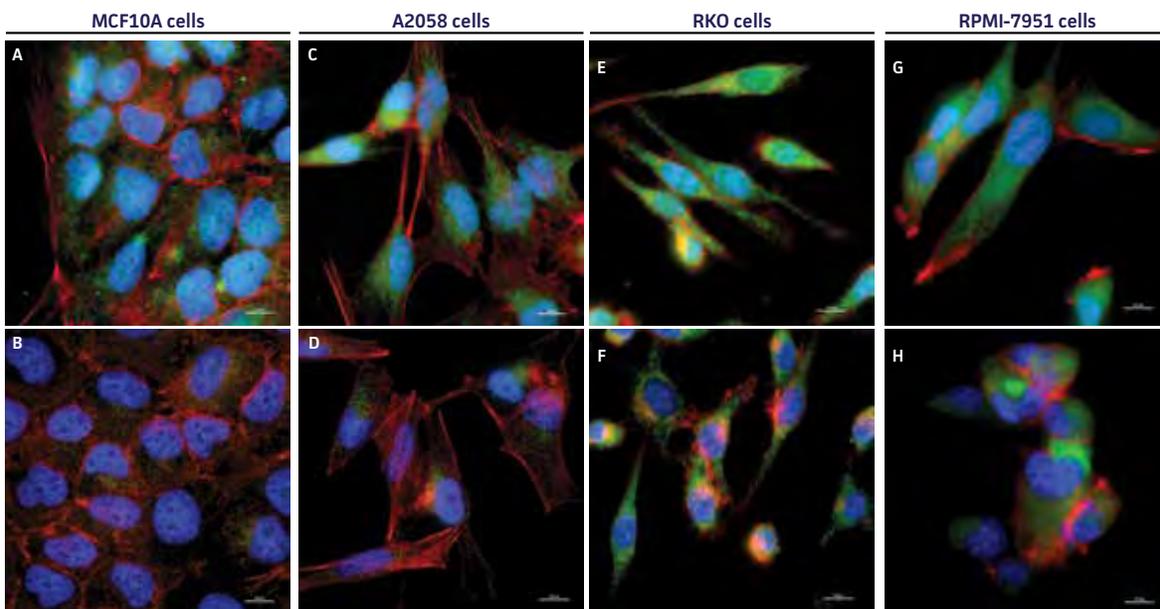
- Somatic mutations analysis was performed by illumina next generation sequencing with greater than 100x coverage. Gene copy number variation analysis was performed by using qBiomarker Copy Number PCR Assay kit from SA Biosciences.
- A list of wild type control cell lines is recommended at the end of this file.

## ATCC® No. TCP-1032™ BRAF Recommended Culture Conditions

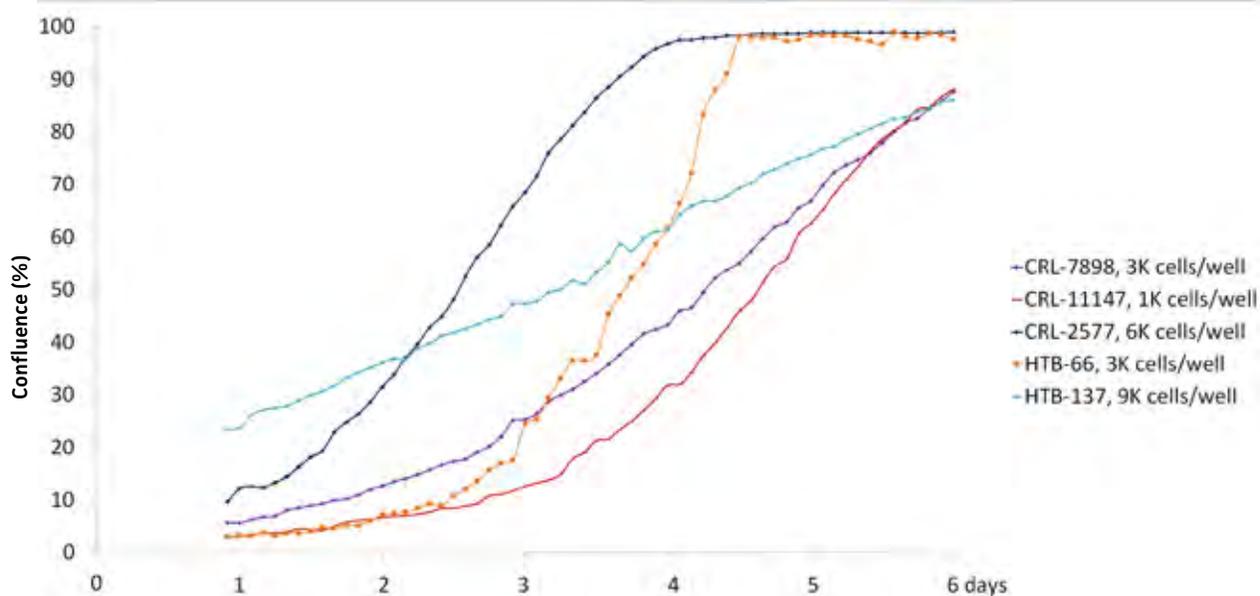
ATCC® No.	Name	Tumor source	Histology	Media	Culture recommendation
CCL-224™	COLO 201	colon	adenocarcinoma	RPMI-1640+10% FBS	seeding density: 2x10 <sup>4</sup> -4x10 <sup>4</sup> cells/mL, subculture every 3-4 days, 1:4 to 1:5 split
CCL-238™	SW1417	colon	adenocarcinoma	Leibovitz's L-15+10% FBS	seeding density: 5x10 <sup>4</sup> -6x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-4 days, 1:3 to 1:5 split
CRL-11147™	A2058	skin	malignant melanoma	DMEM+10 % FBS	seeding density: 5x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-5 days, 1:5-1:10 split
CRL-2577™	RKO	colon	carcinoma	EMEM+10% FBS	seeding density: 2x10 <sup>4</sup> -4x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-4 days, 1:8 to 1:12 split
CRL-7898™	A101D	skin	malignant melanoma	DMEM+10% FBS	seeding density: 3x10 <sup>4</sup> -4x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 4-5 days, 1:4 split
HTB-123™	DU4475	breast	ductal carcinoma	RPMI-1640+10% FBS	seeding density: 2x10 <sup>5</sup> cells/mL, subculture every 4-5 days, 1:3 to 1:5 split
HTB-137™	Hs 695T	skin	malignant melanoma	EMEM+10% FBS	seeding density: 4x10 <sup>4</sup> -5x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 4-5 days, 1:3 to 1:5 split
HTB-66™	RPMI-7951	skin	malignant melanoma	EMEM+10% FBS	seeding density: 2.0x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 5 days, 1:5 to 1:7 split



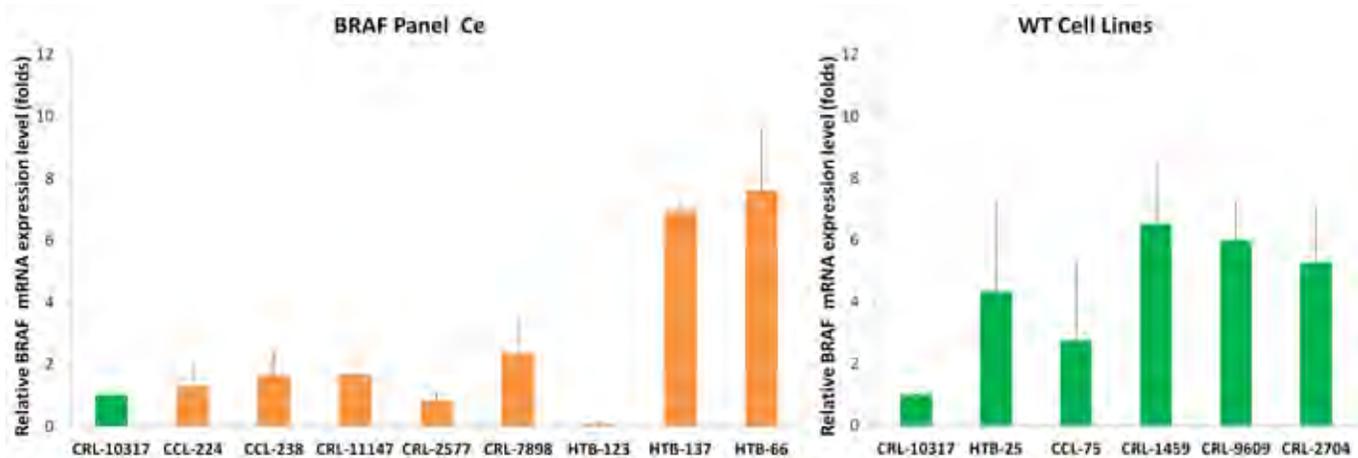
**Figure 1.** Cell morphology of the eight tumor cell lines in the BRAF Genetic Alteration Cell Panel. Cells were maintained in ATCC recommended culture condition. Cell morphology was observed under Nikon microscopy, and images of the indicated cell lines were captured by Olympus digital camera.



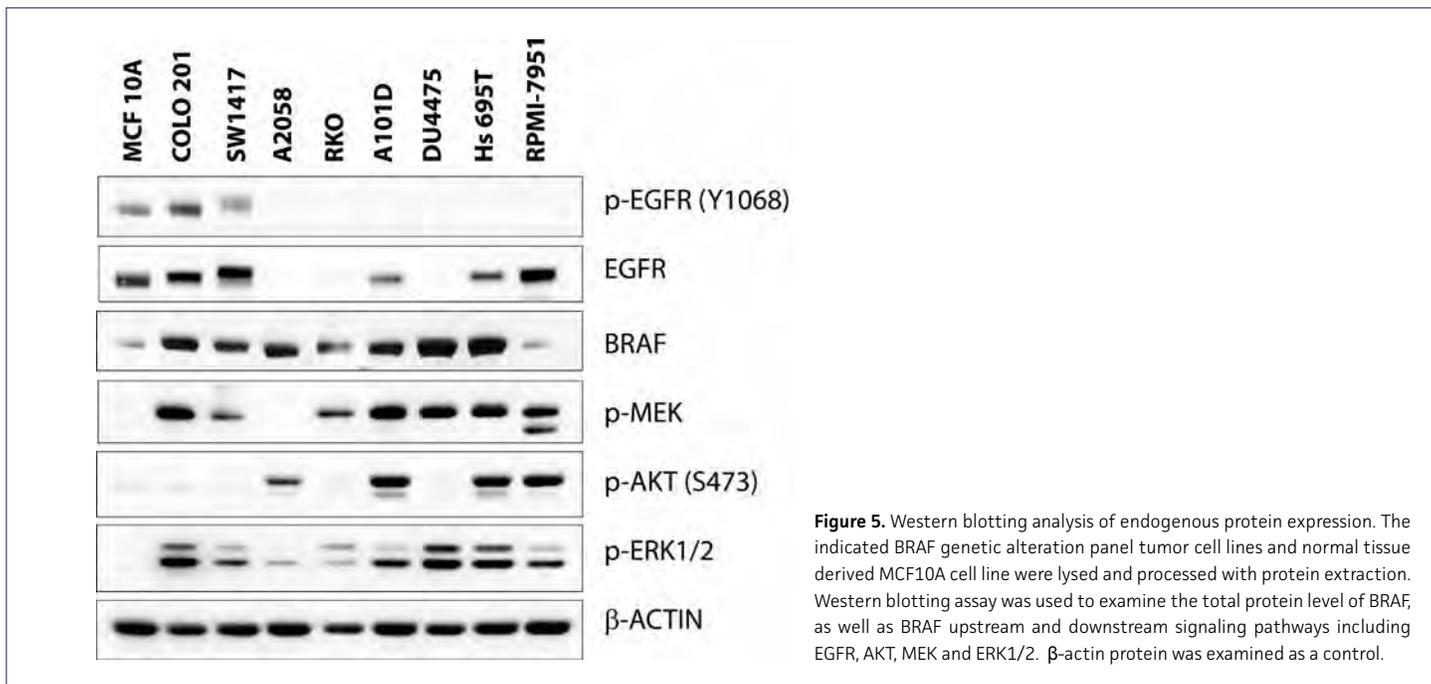
**Figure 2.** The recommended BRAF WT control cells (A,B) and indicated BRAF genetic alteration cells (C-H) were grown on collagen coated coverslips. Cells were fixed with 4% paraformaldehyde. BRAF was stained with BRAF primary antibody and Alexa Fluor 488 secondary antibody (green) in the upper row (A, C, E, G). The downstream signaling MEK was stained with phospho- MEK primary antibody and Alexa Fluor 488 secondary antibody (green) in the lower row (B, D, F, H). Nuclei of the cells were visualized with Hoechst 33342 (blue). F-actin was visualized with phalloidin Alexa Fluor 594 (red). Multichannel merged images of indicated protein from above, F-actin and nuclei were shown as three-color images.



**Figure 3.** Cell growth kinetics. The indicated genetic alteration cells were cultured in ATCC recommended media, and plated in a 96 well plate at 1000 cells/well to 9000 cells/well. The cell growth kinetics were constantly monitored for 6 days by using a label-free automated IncuCyte live-cell imaging system (Essen Bioscience).



**Figure 4.** Real time PCR analysis of mRNA levels. The mRNA expression level of BRAF and 36B4 were determined by real time quantitative PCR. Relative BRAF mRNA expression of indicated BRAF genetic alteration panel tumor cell lines were calculated by comparing to normal tissue derived MCF10A cells after normalization to the housekeeping gene 36B4. The data of recommended WT control cell lines was also provided. Tumor cell lines within the panel were shown as orange, and WT control cell lines were shown as green.



#### Recommended Control Cell Lines

ATCC® No.	Name	Tissue source	Cell type	Histology
HTB-25™	MDA-MB-175-VII	breast	epithelial	ductal carcinoma
CRL-10317™	MCF 10A	breast	epithelial	normal
CCL-75™	WI-38	lung	fibroblast	normal
CRL-9609™	BEAS-2B	lung	epithelial	normal
CRL-1459™	CCD-18Co	colon	fibroblast	normal
CRL-2704™	C13589	haematopoietic and lymphoid tissue	B lymphoblast	normal

# EGFR GENETIC ALTERATION PANEL (ATCC® NO. TCP-1027™)

EGFR/ERBB1/HER1 and ERBB2/EGFR2/HER2 are members of the ErbB super family of receptor tyrosine kinases. These receptors bind multiple EGF family-member ligands to initiate signaling cascades critical for an array of cellular processes, such as proliferation, differentiation, survival, metabolism, and migration. Mutations and amplifications of EGFR or family members have occurred frequently in human cancers.

The EGFR Genetic Alteration Cell Panel (ATCC® No. TCP-1027™) is composed of eleven select human tumor cell lines, derived from a variety of common cancer types that carry hotspot mutations or gene copy number amplification within the EGFR or ERBB2 gene. The EGFR and ERBB2 status of each cell line has been sequenced and validated by ATCC. This panel is useful for performing EGFR pathway research, developing EGFR inhibitors, or evaluating novel anti-cancer therapeutics.

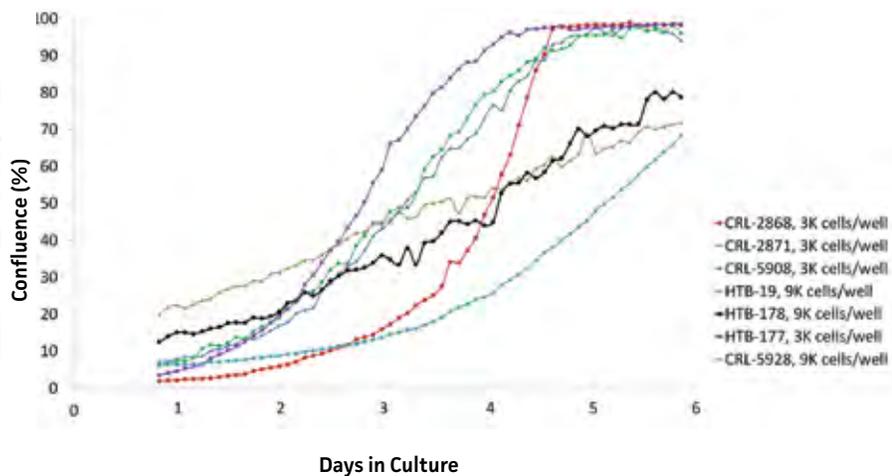
## ATCC® No. TCP-1027™ EGFR Genetic Alteration Cell Panel

ATCC® No.	Name	Gene	DNA Change	Zygoty	Amino acid Change	EGFR copy number variation	ERBB2 copy number variation	Tumor source
CRL-2868™	HCC827	EGFR	c.2236_2250delGAATTAAGAGAAGCA	Heterozygous	p.ELREA746del	amplification	–	lung
CRL-2871™	HCC4006	EGFR	c.2236_2244delGAATTAAGA	Heterozygous	p.ELR746del	–	–	lung
CCL-231™	SW48	EGFR	c.2155G>A	Heterozygous	p.G719S	–	–	colon
CRL-5908™	NCI-H1975	EGFR	c.2369C>T c.2573T>G	Heterozygous Heterozygous	p.T790M p.L858R	–	–	lung
HTB-132™	MDA-MB-468	EGFR	–	–	–	amplification	–	breast
HTB-19™	BT-20	EGFR	–	–	–	amplification	–	breast
HTB-178™	NCI-H596	EGFR	–	–	–	amplification	–	lung
HTB-177™	NCI-H460	EGFR	–	–	–	–	–	lung
CRL-5928™	NCI-H2170	ERBB2	–	–	–	–	amplification	lung
HTB-20™	BT-474	ERBB2	–	–	–	–	amplification	breast
HTB-27™	MDA-MB-361	ERBB2	–	–	–	–	amplification	breast

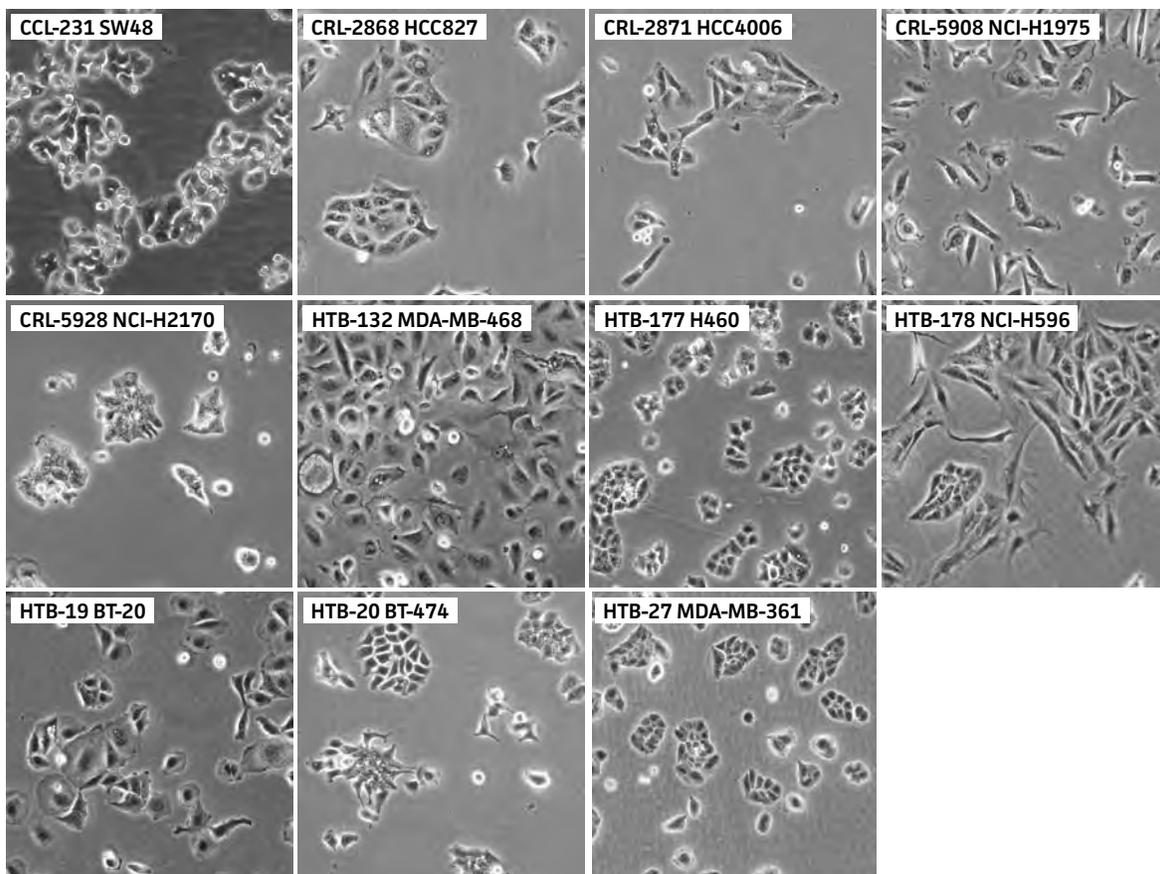
- Somatic mutations analysis was performed by illumina next generation sequencing with greater than 100x coverage. Gene copy number variation analysis was performed by using qBiomarker Copy Number PCR Assay kit from SA Biosciences.
- A list of wild type control cell lines is recommended at the end of this file.

## ATCC® No. TCP-1027™ Recommended Culture Conditions

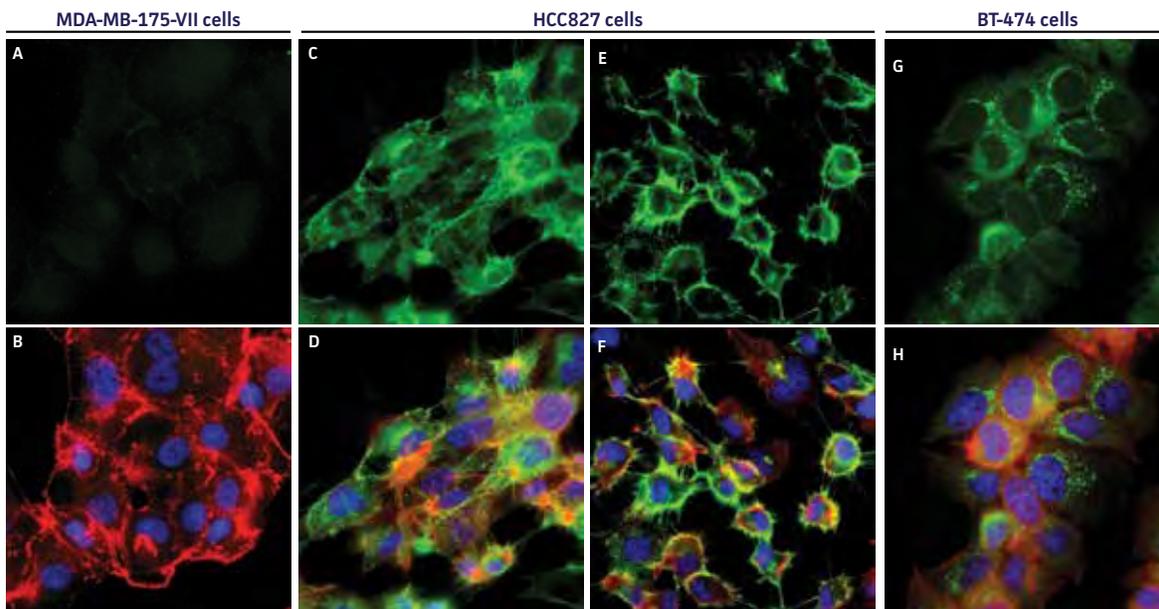
ATCC® No.	Name	Tumor source	Histology	Media	Culture recommendation
CRL-2868™	HCC827	lung	adenocarcinoma	RPMI-1640 + 10% FBS	seeding density: 2x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-4 days, 1:5 split
CRL-2871™	HCC4006	lung	adenocarcinoma	RPMI-1640 + 10% FBS	seeding density: 2x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 4 days, 1:5 split
CCL-231™	SW48	colon	adenocarcinoma	Leibovitz's L-15 + 10% FBS	seeding density: 5x10 <sup>4</sup> - 1x10 <sup>5</sup> cells/cm <sup>2</sup> , subculture every 4-5 days, 1:5 split
CRL-5908™	NCI-H1975	lung	non small cell carcinoma	RPMI-1640 + 10% FBS	seeding density: 4x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 4-5 days, 1:5 split
HTB-132™	MDA-MB-468	breast	adenocarcinoma	Leibovitz's L-15 + 10% FBS	seeding density: 2x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 4-5 days, 1:5 split
HTB-19™	BT-20	breast	carcinoma	RPMI-1640+ 10% FBS	seeding density: 2.0x10 <sup>4</sup> -4.0x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 2-5 days, 1:2 to 1:5 split
HTB-178™	NCI-H596	lung	adenosquamous carcinoma	EMEM + 10% FBS	seeding density: 2.0x10 <sup>4</sup> -4.0x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 5-7 days, 1:2 to 1:4 split
HTB-177™	NCI-H460	lung	large cell carcinoma	RPMI-1640 + 10% FBS	seeding density: 6x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-4 days, 1:10 split
CRL-5928™	NCI-H2170	lung	squamous cell carcinoma	RPMI-1640 + 10% FBS	seeding density: 6x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-4 days, 1:5 split
HTB-20™	BT-474	breast	ductal carcinoma	HybriCare + 10% FBS	seeding density: 3.0 x 10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 6-7 days, 1:6 to 1:9 split
HTB-27	MDA-MB-361	breast	adenocarcinoma	Leibovitz's L-15 + 20% FBS	seeding density: 6x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 4-5 days, 1:5 split



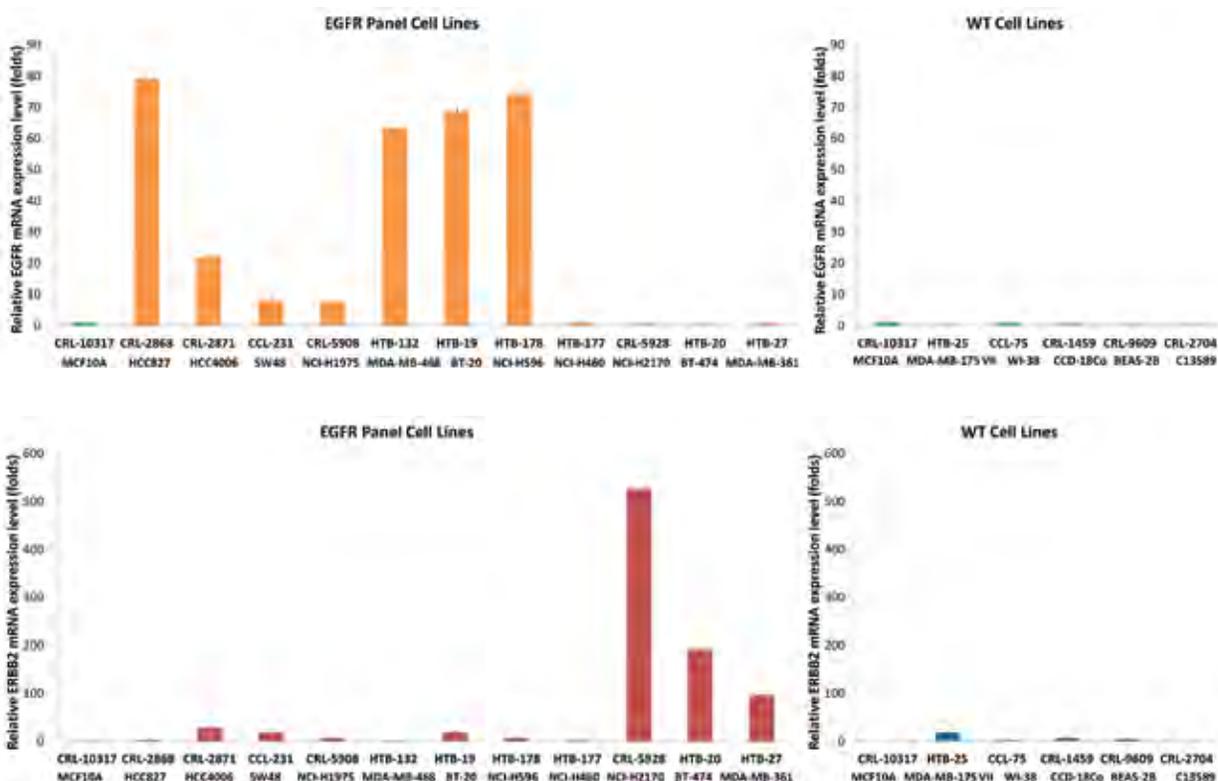
**Figure 1.** Cell-growth kinetics. The indicated cell lines were cultured in ATCC recommended media, and plated in a 96 well plate at 1,000 – 9,000 cells/well. Cell-growth kinetics were constantly monitored for 6 days using a label-free automated IncuCyte live-cell imaging system (Essen Bioscience).



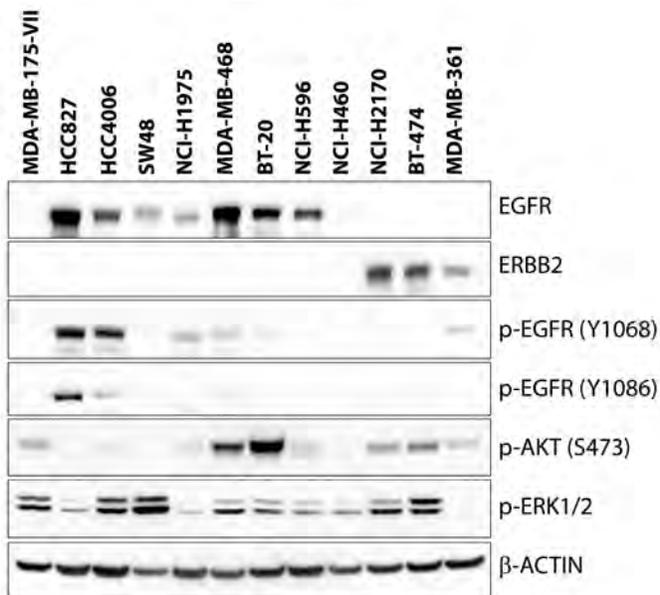
**Figure 2.** Cell morphology of the eleven tumor cell lines in the EGFR Genetic Alteration Cell Panel. Cells were maintained in ATCC recommended culture conditions. Cell morphology was observed under Nikon™ microscopy, and images of the indicated cell lines were captured using an Olympus® digital camera.



**Figure 3.** Immunofluorescence staining of EGFR and ERBB2. The indicated EGFR wild-type (A, B) and EGFR mutant (C-F) or ERBB2 mutant (G-H) cell lines were grown on collagen-coated coverslips and fixed with 4% paraformaldehyde. EGFR wild-type cells were stained with an antibody against EGFR (A) or an EGFR antibody and the F-actin label, phalloidin (B, green and red, respectively). Constitutively-active EGFR-mutant HCC827 cells were stained with an antibody against EGFR (C) or an EGFR antibody and phalloidin (D, green and red, respectively). The HCC827 cells were also stained with an antibody against p-EGFR (E) or p-EGFR and phalloidin (F, green and red, respectively) to show that EGFR is constitutively activated in these cells. ERBB2-mutant cells were stained with an antibody against ERBB2 (G) or an ERBB2 antibody and phalloidin (H, green and red, respectively). Nuclei are stained with Hoechst 33342 (blue) in the multi-channel images in the lower row.



**Figure 4.** Real-time qPCR analysis of mRNA levels. The mRNA expression level of EGFR and ERBB2 were determined by real time quantitative PCR. Relative EGFR (orange and green bars, upper panel) and ERBB2 (red and blue bars, lower panel). mRNA expression for the indicated cell lines was calculated by normalizing their levels to the wild-type cell line MCF10A (Set to 1) and the housekeeping gene 36B4.



**Figure 5.** Western blotting analysis of endogenous protein expression. The indicated cell lines were lysed and processed to extract protein. Western blotting was used to examine the total protein level and phosphorylation of EGFR, as well as markers of downstream EGFR signaling pathways such as p-AKT and p-ERK.  $\beta$ -actin protein was included as a loading-control.

#### Recommended Control Cell Lines

ATCC <sup>®</sup> No.	Name	Tissue Source	Cell Type	Histology
HTB-25 <sup>™</sup>	MDA-MB-175-VII	breast	epithelial	ductal carcinoma
CRL-10317 <sup>™</sup>	MCF 10A	breast	epithelial	normal
CCL-75 <sup>™</sup>	WI-38	lung	fibroblast	normal
CRL-9609 <sup>™</sup>	BEAS-2B	lung	epithelial	normal
CRL-1459 <sup>™</sup>	CCD-18Co	colon	fibroblast	normal
CRL-2704 <sup>™</sup>	C13589	hematopoietic and lymphoid	B lymphoblast	normal

# ERK GENETIC ALTERATION CELL PANEL (ATCC® NO. TCP-1033™)

ERK1 and ERK2 are important members of the mitogen-activated protein kinases (MAPKs) family, which controls a broad range of cellular activities and physiological processes such as proliferation, cell survival, migration, morphology determination and oncogenic transformation. The MAPK1 gene encodes the ERK2 protein, and the MAPK3 gene encodes the ERK1 protein. ERK1 and ERK2 can be activated by upstream RAS-RAF-MEK signaling. Elevated and constitutive activation of these two proteins has been detected in various common cancer types. Mutations in the MAPK1 and MAPK3 genes have been observed in a subset of human tumors by next generation sequencing. The ERK genetic alteration cell panel (ATCC® No. TCP-1033™) is composed of 7 selected human tumor cell lines from various common cancer types that carry COSMIC database validated mutations within MAPK1 and MAPK3 genes. This panel of cell lines also encompasses various basal levels of phosphor-ERK1/2. The MAPK1 and MAPK3 gene alteration status of each cell line has been sequenced and validated by ATCC. This panel is useful for studying the impact of MAPK mutants on ERK protein bio-functions, RAS-RAF-ERK pathway research, as well as developing ERK inhibitors as anti-cancer therapeutics.

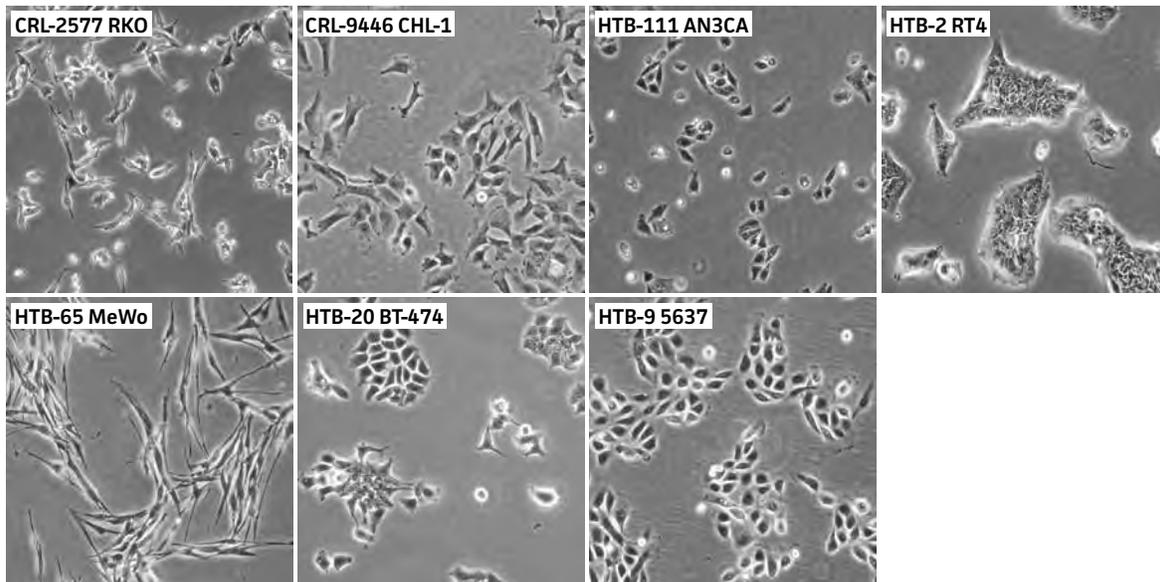
## ATCC® No. TCP-1033™ ERK Genetic Alteration Cell Panel

ATCC® No.	Name	Gene	DNA Change	Zygoty	Amino acid Change	Tumor source
CRL-2577™	RKO	MAPK3	c.288C>T	Heterozygous	p.R96R	colon
CRL-9446™	CHL-1	MAPK3	c.682A>G	Homozygous	p.I228V	skin
HTB-111™	AN3 CA	MAPK3	c.1117C>T	Heterozygous	p.P373S	endometrium
HTB-2™	RT4	MAPK3	c.327G>A	Heterozygous	p.A109A	urinary bladder
HTB-65™	MeWo	MAPK3	c.736C>T	Heterozygous	p.P246S	skin
HTB-20™	BT-474	MAPK1	c.183C>G	Heterozygous	p.H61Q	breast
HTB-9™	5637	MAPK1	c.236G>A	Heterozygous	p.R79K	urinary bladder

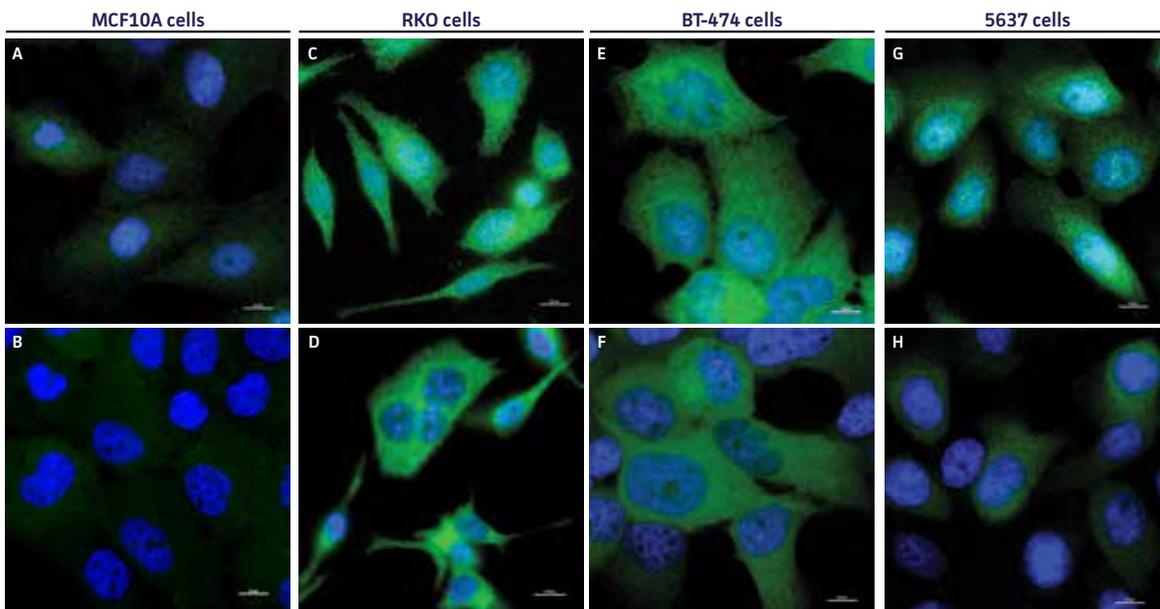
- Somatic mutations analysis was performed by illumina next generation sequencing with greater than 100x coverage. Gene copy number variation analysis was performed by using qBiomarker Copy Number PCR Assay kit from SA Biosciences.
- A list of wild type control cell lines is recommended at the end of this file.

## ATCC® No. TCP-1033™ ERK Recommended Culture Conditions

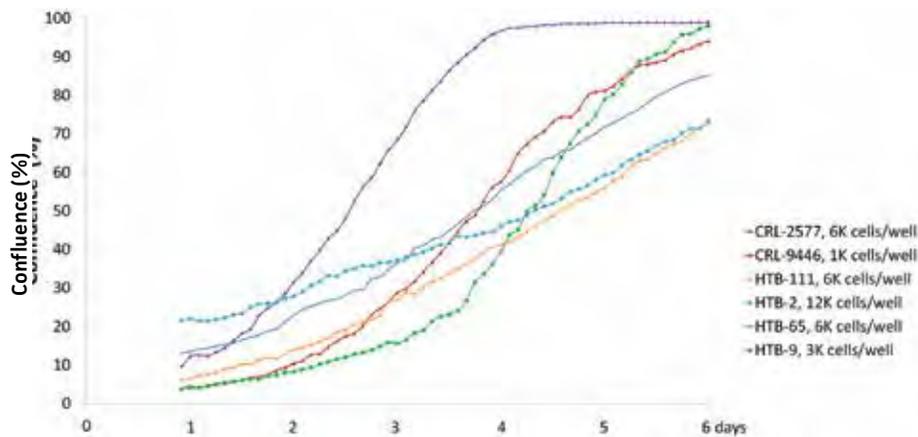
ATCC® No.	Name	Tumor source	Histology	Media	Culture recommendation
CRL-2577™	RKO	colon	carcinoma	EMEM+10% FBS	seeding density: $2 \times 10^4$ - $4 \times 10^4$ cells/cm <sup>2</sup> , subculture every 3-4 days, 1:8 to 1:12 split
CRL-9446™	CHL-1	skin	malignant melanoma	DMEM + 10% FBS	seeding density: $1.0 \times 10^4$ - $2.5 \times 10^4$ cells/cm <sup>2</sup> , subculture every 2-4 days, 1:6 to 1:10 split
HTB-111™	AN3 CA	endometrium	adenocarcinoma	EMEM+10% FBS	seeding density: $3.0 \times 10^4$ cells/cm <sup>2</sup> , subculture every 7 days, 1:5 to 1:7 split
HTB-2™	RT4	urinary bladder	transitional cell carcinoma	McCoy's 5A	seeding density: $3.0 \times 10^4$ - $7 \times 10^4$ cells/cm <sup>2</sup> , subculture every 3-6 days, 1:2 to 1:5 split
HTB-65™	MeWo	skin	malignant melanoma	EMEM+10% FBS	seeding density: $3.5 \times 10^4$ - $7 \times 10^4$ cells/cm <sup>2</sup> , subculture every 3-6 days, 1:2 to 1:5 split
HTB-20™	BT-474	breast	ductal carcinoma	Hybicare + 10% FBS	seeding density: $3.0 \times 10^4$ - $1.0 \times 10^5$ cells/cm <sup>2</sup> , subculture every 4-7 days, 1:2 to 1:9 split
HTB-9™	5637	urinary bladder	carcinoma	RPMI-1640 + 10% FBS	seeding density: $1.0 \times 10^4$ - $3 \times 10^4$ cells/cm <sup>2</sup> , subculture every 2-4 days, 1:5 to 1:10 split



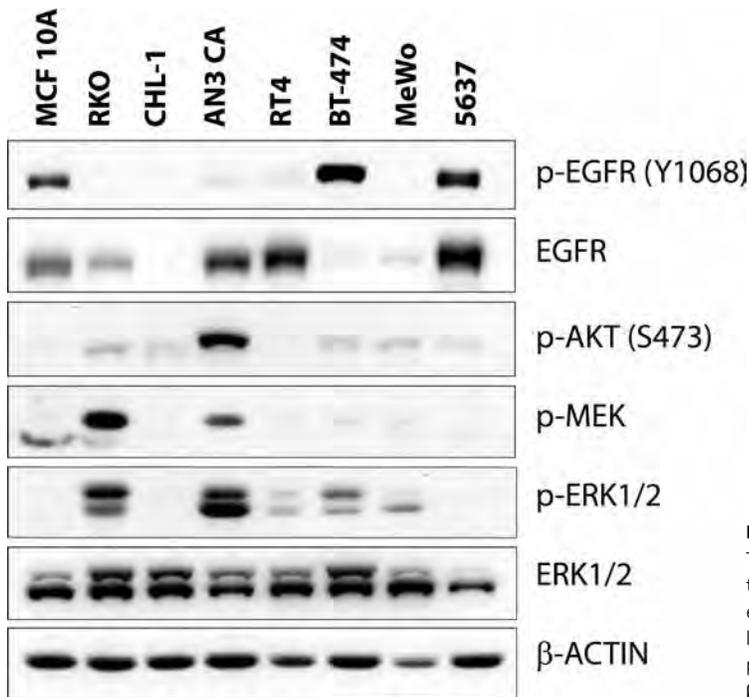
**Figure 1.** Cell morphology of the seven tumor cell lines in the ERK Genetic Alteration Cell Panel. Cells were maintained in ATCC recommended culture conditions. Cell morphology was observed under Nikon microscopy, and images of the indicated cell lines were captured by an Olympus digital camera.



**Figure 2.** Immunofluorescence staining. The recommended ERK WT control cells (A,B) and indicated ERK genetic alteration cells (C-H) were grown on collagen coated coverslips. Cells were fixed with 4% paraformaldehyde. Total ERK was stained with ERK1/2 primary antibody and Alexa Fluor 488 secondary antibody (green) in the upper row (A, C, E, G). The phospho-ERK1/2 was stained with phospho-ERK1/2 primary antibody and Alexa Fluor 488 secondary antibody (green) in the lower row (B, D, F, H). Nuclei of the cells were visualized with Hoechst 33342 (blue). Multichannel merged images of indicated protein from above.



**Figure 3.** Cell growth kinetics. The indicated genetic alteration panel tumor cell lines were cultured in ATCC recommended media, and plated in 96 well plate at 1000 cells/well - 9000 cells/well seeding density. The cell growth kinetics were constantly monitored for 6 days by using a label-free automated IncuCyte live-cell imaging system (Essen Bioscience).



**Figure 4.** Western blotting analysis of endogenous protein expression. The indicated ERK genetic alteration panel tumor cell lines normal tissue derived MCF10A cell line were lysed and processed with protein extraction. Western blotting assay was used to examine the protein level and phosphorylation of ERK1/2, the ERK upstream signaling pathways including EGFR and MEK, as well as phosphorylation of AKT.  $\beta$ -actin protein was examined as a control.

#### Recommended Control Cell Lines

ATCC® No.	Name	Tissue source	Cell Type	Histology
HTB-25™	MDA-MB-175-VII	breast	epithelial	ductal carcinoma
CRL-10317™	MCF 10A	breast	epithelial	normal
CCL-75™	WI-38	lung	fibroblast	normal
CRL-9609™	BEAS-2B	lung	epithelial	normal
CRL-1459™	CCD-18Co	colon	fibroblast	normal
CRL-2704™	C13589	haematopoietic and lymphoid tissue	B lymphoblast	normal

# FGFR GENETIC ALTERATION CELL PANEL (ATCC® NO. TCP-1034™)

The Fibroblast Growth Factor Receptor (FGFRs) are transmembrane tyrosine kinase receptors that induce intracellular tyrosine kinase activity when bound to their ligand, Fibroblast Growth Factor. The FGFRs play crucial roles in development and cell growth. Upregulation of the FGF/FGFR signaling pathway leads to induction of mitogenic and survival signals, as well as promoting epithelial-mesenchymal transition, invasion and tumor angiogenesis. Amplification or activation of either the FGFR1 or FGFR2 genes has been linked to several cancer types such as lung cancer, breast cancer and gastric cancer. The FGFR genetic alteration cell panel (ATCC® No. TCP-1034™) is composed of eight selected human tumor cell lines from common cancer types that carry various gene copy number amplification within the FGFR1 or FGFR2 genes. The FGFR1 or FGFR2 status of each cell line has been validated by ATCC. This panel is useful for FGFR pathway research and FGFR inhibitors anti-cancer drug discovery.

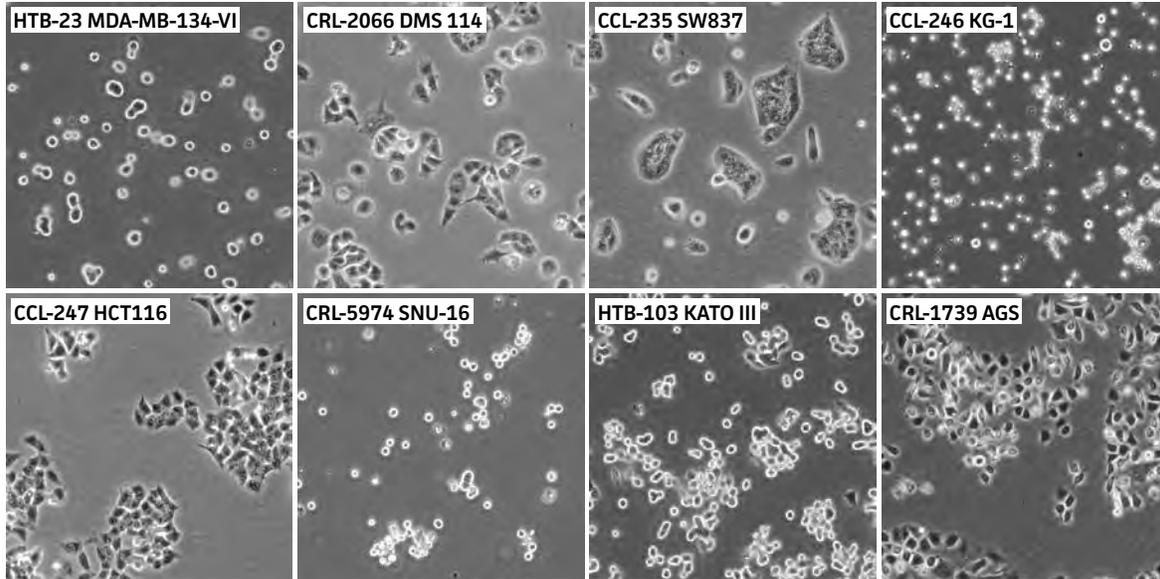
## ATCC® No. TCP-1034™ FGFR Genetic Alteration Cell Panel

ATCC® No.	Name	Gene	FGFR1 copy number variation	FGFR2 copy number variation	Tumor source
HTB-23™	MDA-MB-134-VI	FGFR1	amplification	–	breast
CRL-2066™	DMS 114	FGFR1	amplification	–	lung
CCL-235™	SW837	FGFR1	slight increase	–	colon
CCL-246™	KG-1	FGFR1	slight increase	–	bone marrow
CCL-247™	HCT116	FGFR1	–	–	colon
CRL-5974™	SNU-16	FGFR2	–	amplification	stomach
HTB-103™	KATO III	FGFR2	–	amplification	stomach
CRL-1739™	AGS	FGFR2	–	–	stomach

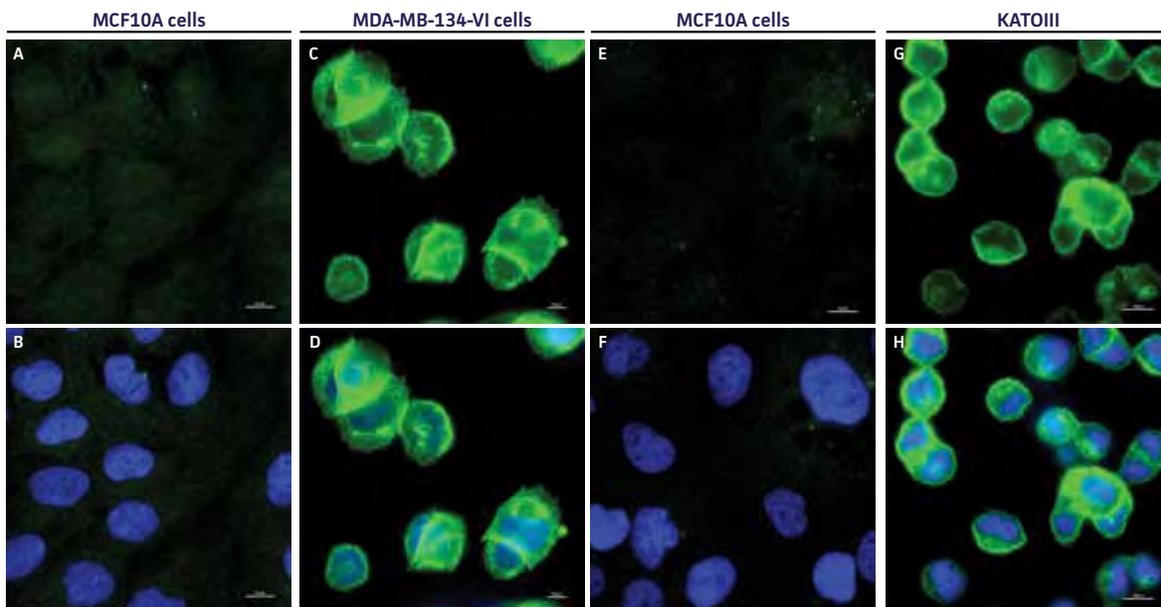
- Somatic mutations analysis was performed by illumina next generation sequencing with greater than 100x coverage. Gene copy number variation analysis was performed by using qBiomarker Copy Number PCR Assay kit from SA Biosciences.
- A list of wild type control cell lines is recommended at the end of this file.

## ATCC® No. TCP-1034™ FGFR Recommended Culture Conditions

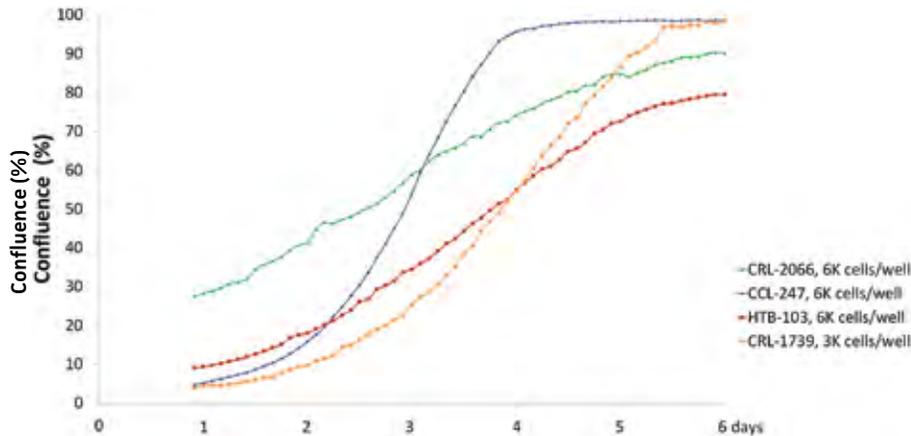
ATCC® No.	Name	Tumor source	Histology	Media	Culture recommendation
HTB-23™	MDA-MB-134-VI	breast	ductal carcinoma	Leibovitz's L-15 + 20% FBS	seeding density: $1.0 \times 10^5$ – $2.0 \times 10^5$ cells/cm <sup>2</sup> , subculture every 10–14 days, 1:2 to 1:4 split
CRL-2066™	DMS 114	lung	small cell carcinoma	Waymouth's MB 752/1 medium+10% FBS	seeding density: $1.0 \times 10^4$ – $2.0 \times 10^4$ cells/cm <sup>2</sup> , subculture every 7–10 days, 1:5 to 1:15 split
CCL-235™	SW837	colon	adenocarcinoma	Leibovitz's L-15 + 10% FBS	seeding density: $3.0 \times 10^4$ – $7.0 \times 10^4$ cells/cm <sup>2</sup> , subculture every 4–7 days, 1:4 to 1:10 split
CCL-246™	KG-1	bone marrow	acute myeloid leukaemia	IMDM + 20% FBS	seeding density: $2 \times 10^5$ cells/mL, subculture every 5–6 days, 1:6 to 1:10 split
CCL-247™	HCT116	colon	colorectal carcinoma	McCoy's 5A+ 10% FBS	seeding density: $5.0 \times 10^3$ – $4.0 \times 10^4$ cells/cm <sup>2</sup> , subculture every 3–6 days, 1:10 to 1:30 split
CRL-5974™	SNU-16	stomach	undifferentiated adenocarcinoma	RPMI-1640 + 10% FBS	seeding density: $2.0$ – $3.0 \times 10^5$ cells/ml, subculture every 3–4 days, 1:3 to 1:5 split
HTB-103™	KATO III	stomach	adenocarcinoma	IMDM + 20% FBS	seeding density: $2.0 \times 10^4$ – $4.0 \times 10^4$ cells/cm <sup>2</sup> , subculture every 4–6 days, 1:8 to 1:15 split
CRL-1739™	AGS	stomach	adenocarcinoma	F-12K + 10% FBS	seeding density: $1.0 \times 10^4$ – $2.0 \times 10^4$ cells/cm <sup>2</sup> , subculture every 3–4 days, 1:5 to 1:8 split



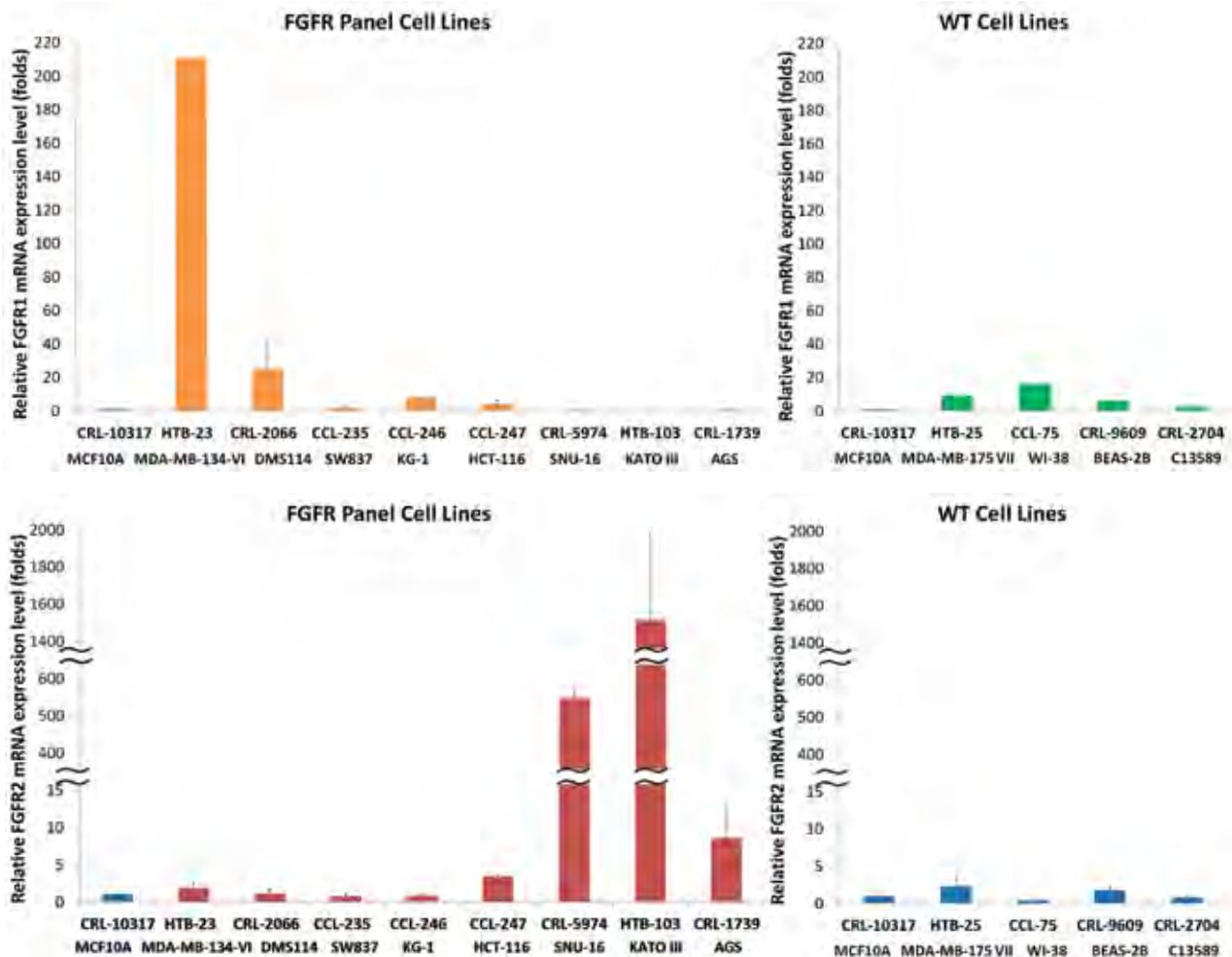
**Figure 1.** Cell morphology of eight tumor cell lines in the FGFR Genetic Alteration Cell Panel. Cells were maintained in ATCC recommended culture conditions. Cell morphology was observed under Nikon microscopy, and images of the indicated cell lines were captured by an Olympus digital camera.



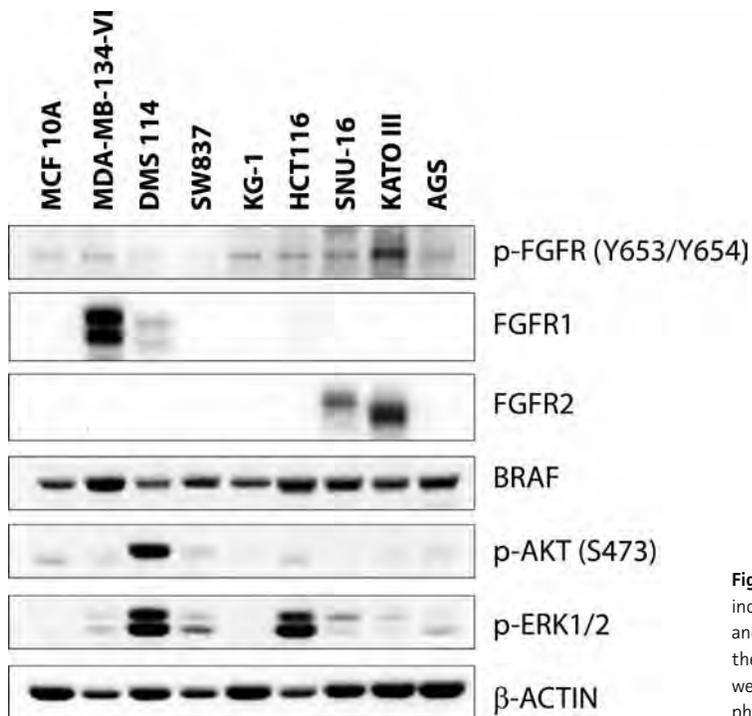
**Figure 2.** Immunofluorescence staining. The recommended ERK WT control cells (A, B, E, F) and indicated FGFR genetic alteration cells (C, D, G, H) were grown on collagen coated coverslips. Cells were fixed with 4% paraformaldehyde. A) -D) total FGFR1 was stained with FGFR1 primary antibody and Alexa Fluor 488 secondary antibody (green). E) -H) total FGFR2 was stained with FGFR2 primary antibody and Alexa Fluor 488 secondary antibody (green). Nuclei of the cells were visualized with Hoechst 33342 (blue) in the lower row. Multichannel merged images of indicated protein from above.



**Figure 3.** Cell growth kinetics. The indicated genetic alteration panel tumor cell lines were cultured in ATCC recommended media, and plated in 96 well plate at 1000 cells/well - 9000 cells/well seeding density. The cell growth kinetics were constantly monitored for 6 days by using a label-free automated IncuCyte live-cell imaging system (Essen Bioscience).



**Figure 4.** Real time PCR analysis of mRNA levels. The mRNA expression level of FGFR1, FGFR2 and 36B4 were determined by real time quantitative PCR. Relative FGFR1 mRNA expression and FGFR2 mRNA expression of indicated FGFR genetic alteration panel tumor cell lines were calculated by comparing to normal tissue derived MCF10A cells after normalization to the housekeeping gene 36B4. The data of recommended WT control cell lines was also provided. Tumor cell lines within the panel were shown as orange, and WT control cell lines were shown as green. Tumor cell lines within the panel were shown as orange or red, and WT control cell lines were shown as green or blue.



**Figure 5.** Western blotting analysis of endogenous protein expression. The indicated FGFR genetic alteration cells and WT MCF10A cell line were lysed and processed to extract protein. Western blotting was used to examine the total protein level of FGFR1 and FGFR2, the phosphorylation of FGFR, as well as FGFR downstream signaling pathways including phosphor-AKT and phosphor-ERK. β-actin protein was examined as a control.

### Recommended Control Cell Lines

ATCC® No.	Name	Tissue source	Cell Type	Histology
HTB-25™	MDA-MB-175-VII	breast	epithelial	ductal carcinoma
CRL-10317™	MCF 10A	breast	epithelial	normal
CCL-75™	WI-38	lung	fibroblast	normal
CRL-9609™	BEAS-2B	lung	epithelial	normal
CRL-1459™	CCD-18Co	colon	fibroblast	normal
CRL-2704™	C13589	hematopoietic and lymphoid tissue	B lymphoblast	normal

# MET GENETIC ALTERATION CELL PANEL (ATCC® NO. TCP-1036™)

MET is a member of the tyrosine kinase receptor family, which leads signal transduction from the extracellular matrix into the cytoplasm by binding to the hepatocyte growth factor (HGF). MET/HGF signaling has been reported to be aberrantly activated in many human cancers. Mutations or amplification of the MET gene has been detected in various human cancers, especially gastric cancer. In addition, amplification of MET correlates with poor prognosis, and plays a role in acquired resistance to EGFR inhibitors of patients with EGFR-mutant tumors. The MET Genetic Alteration Cell Panel (ATCC® No. TCP-1036™) is composed of five human tumor cells that carry various degrees of MET gene copy number changes. The MET status of each cell line has been validated by ATCC. This panel is useful for studying bio-functions of MET and MET amplification, as well as tyrosine kinase inhibitor anti-cancer drug discovery.

## ATCC® No. TCP-1036™ MET Genetic Alteration Cell Panel

ATCC® No.	Name	Gene	Copy number variation	Tumor source
CRL-5973™	SNU-5	MET	amplification	stomach
HTB-135™	Hs 746T	MET	amplification	stomach
CRL-1585™	C32	MET	slight increase	skin
CRL-2351™	AU565	MET	slight increase	breast
CRL-5822™	NCI-N87	MET	–	stomach

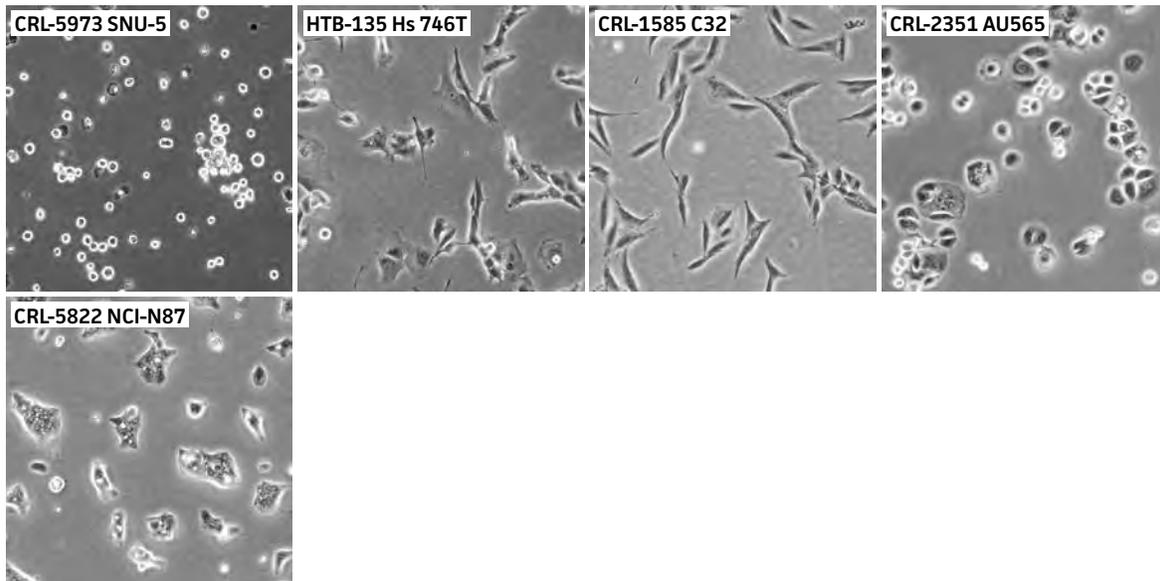
- Somatic mutations analysis was performed by illumina next generation sequencing with greater than 100x coverage. Gene copy number variation analysis was performed by using qBiomarker Copy Number PCR Assay kit from SA Biosciences.
- A list of wild type control cell lines is recommended at the end of this file.

## ATCC® No. TCP-1036™ MET Recommended Culture Conditions

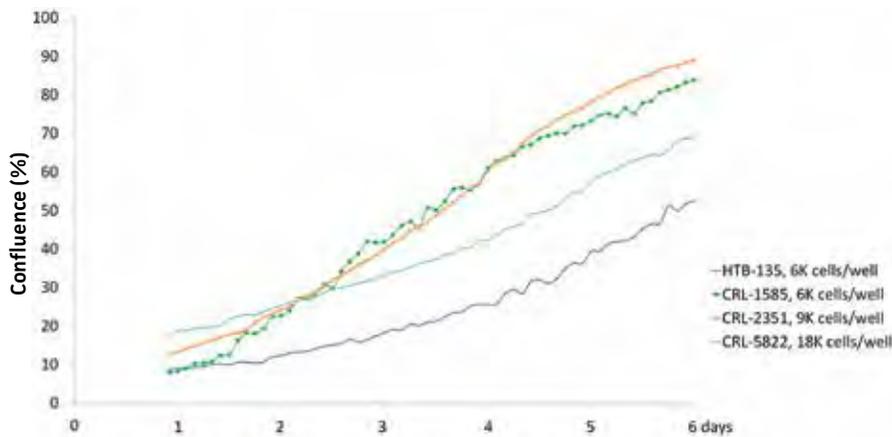
ATCC® No.	Name	Tumor source	Histology	Media	Culture recommendation
CRL-5973™	SNU-5	stomach	undifferentiated adenocarcinoma	DMEM + 10% FBS	seeding density: $2.0 \times 10^4$ – $4.0 \times 10^4$ viable cells/cm <sup>2</sup> , subculture every 7 days. 1:3 split
HTB-135™	Hs 746T	stomach	carcinoma	DMEM + 10% FBS	seeding density: $4.0 \times 10^4$ viable cells/cm <sup>2</sup> , subculture every 7 days. 1:3 split
CRL-1585™	C32	skin	malignant melanoma	EMEM + 10% FBS	seeding density: $4.0 \times 10^4$ cells/cm <sup>2</sup> , subculture every 5 days, 1:8 split
CRL-2351™	AU565	breast	carcinoma	RPMI-1640 + 10% FBS	seeding density: $4.0 \times 10^4$ viable cells/cm <sup>2</sup> , subculture every 4 days. 1:3 split
CRL-5822™	NCI-N87	stomach	carcinoma	RPMI-1640 + 10% FBS	seeding density: $5.0 \times 10^4$ – $1.0 \times 10^5$ viable cells/cm <sup>2</sup> , subculture every 5–7 days. 1:3 to 1:5 split

## Recommended Control Cell Lines

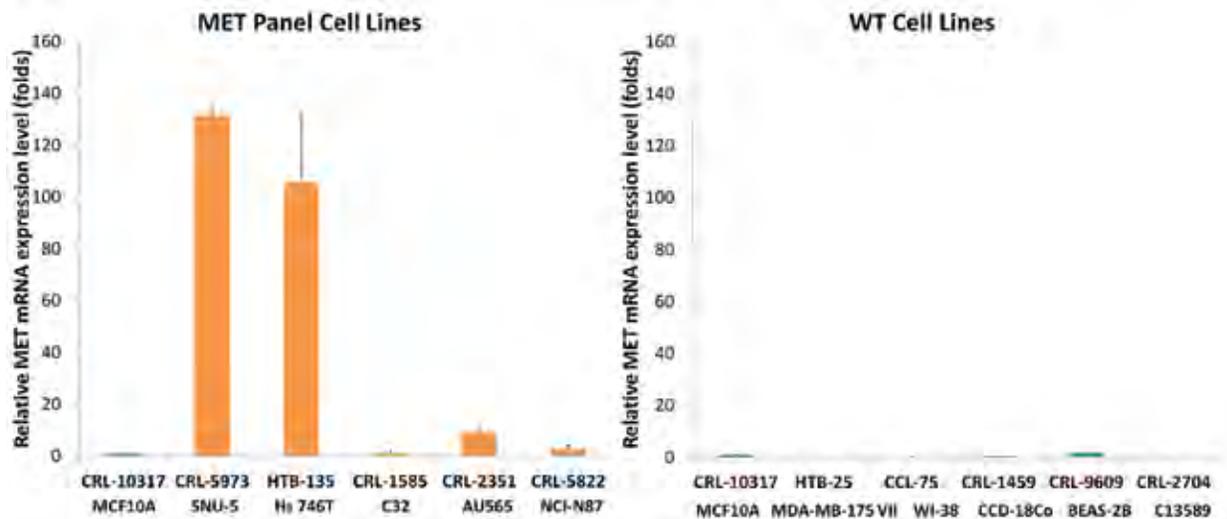
ATCC® No.	Name	Tissue source	Cell Type	Histology
HTB-25™	MDA-MB-175-VII	breast	epithelial	ductal carcinoma
CRL-10317™™	MCF 10A	breast	epithelial	normal
CCL-75™	WI-38	lung	fibroblast	normal
CRL-9609™	BEAS-2B	lung	epithelial	normal
CRL-1459™	CCD-18Co	colon	fibroblast	normal
CRL-2704™	C13589	haematopoietic and lymphoid tissue	B lymphoblast	normal



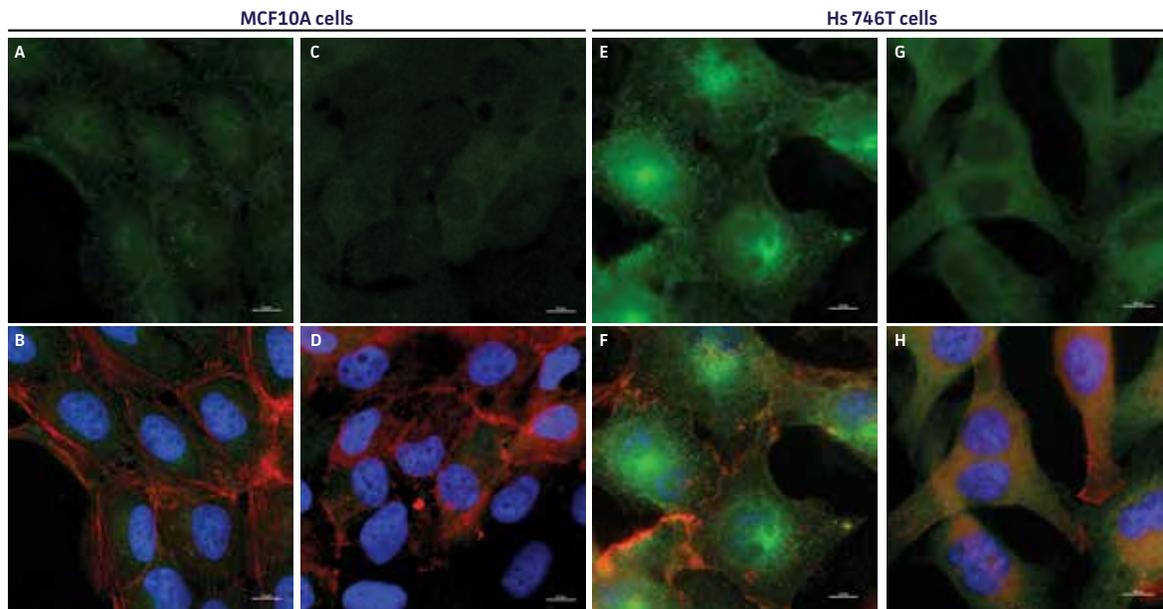
**Figure 1.** Cell morphology of the five tumor cell lines in the MET Genetic Alteration Cell Panel. Cells were maintained in ATCC recommended culture condition. Cell morphology was observed under Nikon microscopy, and images of the indicated cell lines were captured by an Olympus digital camera.



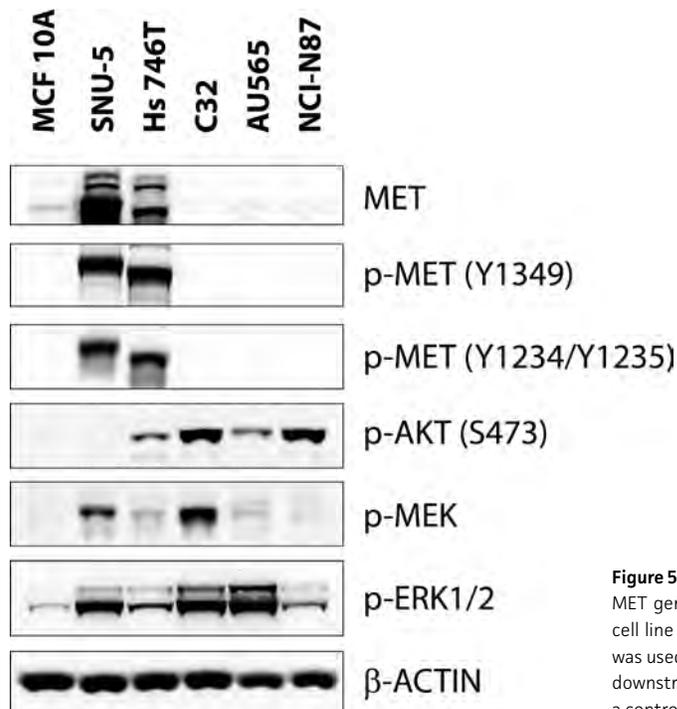
**Figure 2.** Cell growth kinetics. The indicated genetic alteration panel tumor cell lines were cultured in ATCC recommended media, and plated in 96 well plate at 1000 cells/well - 9000 cells/well seeding density. The cell growth kinetics were constantly monitored for 6 days by using a label-free automated IncuCyte live-cell imaging system (Essen Bioscience).



**Figure 3.** Real time PCR analysis of mRNA levels. The mRNA expression level of MET and 36B4 were determined by real time quantitative PCR. Relative MET mRNA expression of indicated MET genetic alteration cells were calculated by comparing to WT MCF10A cells after normalization to the housekeeping gene 36B4. The data of recommended WT control cell lines was also provided. Tumor cell lines within the panel were shown as orange, and WT control cell lines were shown as green.



**Figure 4.** Immunofluorescence staining. The recommended MET WT control cells (A-D) and indicated MET genetic alteration cells (E-H) were grown on collagen coated coverslips. Cells were fixed with 4% paraformaldehyde. A), B), E) and F): MET was stained with MET primary antibody and Alexa Fluor 488 secondary antibody (green). C), D), G) and H): The downstream signaling phosphor-ERK1/2 was stained with phosphor-ERK1/2 primary antibody and Alexa Fluor 488 secondary antibody (green). Nuclei of the cells were visualized with Hoechst 33342 (blue). F-actin was visualized with phalloidin Alexa Fluor 594 (red). Multichannel merged images of indicated protein from above, F-actin and nuclei were shown as three-color images.



**Figure 5.** Western blotting analysis of endogenous protein expression. The indicated MET genetic alteration panel tumor cell lines and normal tissue derived MCF10A cell line were lysed and processed with protein extraction. Western blotting assay was used to examine the protein level and phosphorylation of PTEN, as well as MET downstream signaling AKT, MEK and ERK1/2.  $\beta$ -actin protein was also examined as a control.

# MYC GENETIC ALTERATION CELL PANEL (ATCC® NO. TCP-1035™)

MYC is an oncogenic transcription factor which is encoded by the c-myc proto-oncogene. MYC regulates target gene expression to affect cell growth, proliferation, biogenesis, cellular metabolism and apoptosis. Mutations or overexpression of c-myc gene has been detected in a wide range of human cancers, especially in lymphomas. The MYC Genetic Alteration Cell Panel (ATCC® No. TCP-1035™) is composed of nine selected human tumor cells that carry c-myc mutations or various degrees of c-myc gene copy number changes. The MYC status of each cell line has been sequenced and validated by ATCC. This panel is useful for studying MYC and its cellular effects on subsequent target genes and pathways in providing a platform for basic research and anti-cancer drug discovery.

## ATCC® No. TCP-1035™ MYC Genetic Alteration Cell Panel

ATCC® No.	Name	Gene	DNA Change	Zygoty	Amino acid change	Copy number variation	Tumor source
CRL-1647™	ST486	MYC	c.152A>T c.214C>A c.328A>C c.593C>T	Homozygous Heterozygous Homozygous Homozygous	p.Q51L p.P72T p.T110P p.A198V	-	ascites
CRL-1648™	CA46	MYC	c.58G>A c.214C>T c.224C>A c.577C>G c.963G>C	Homozygous Homozygous Heterozygous Heterozygous Heterozygous	p.V20I p.P72S p.P75H p.L193V p.Q321H	-	haematopoietic and lymphoid tissue
HTB-62™	P3HR-1	MYC	c.80A>C c.214C>T c.339G>C c.162G>C	Heterozygous Heterozygous Heterozygous Heterozygous	p.Y27S p.P72S p.Q113H p.E54D	-	ascites
CRL-5974™	SNU-16	MYC	-	-	-	amplification	stomach
HTB-175™	NCI-H82	MYC	-	-	-	amplification	lung
CRL-2081™	MSTO-211H	MYC	-	-	-	amplification	pleura
HTB-171™	NCI-H446	MYC	-	-	-	amplification	lung
CCL-240™	HL-60	MYC	-	-	-	amplification	peripheral blood
CRL-9068™	NCI-H929	MYC	-	-	-	-	bone marrow

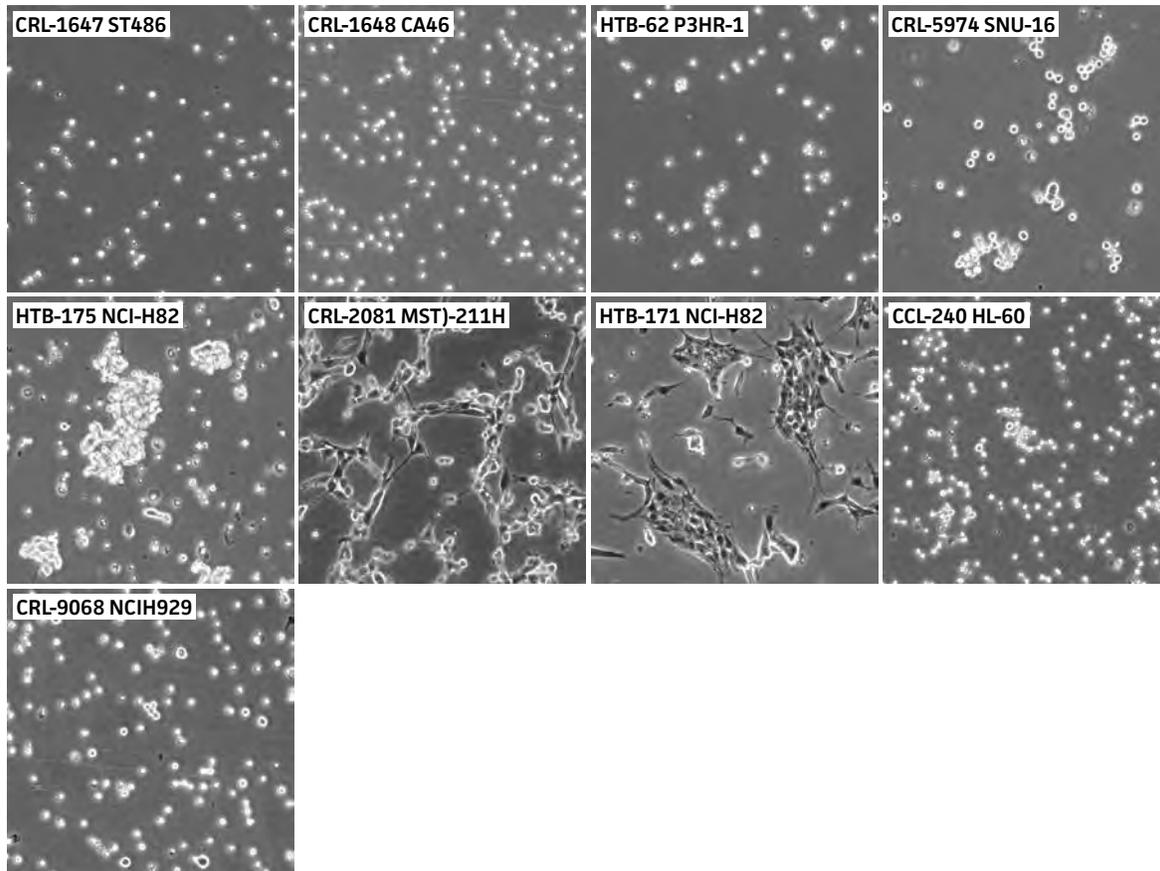
- Somatic mutations analysis was performed by illumina next generation sequencing with greater than 100x coverage. Gene copy number variation analysis was performed by using qBiomarker Copy Number PCR Assay kit from SA Biosciences.
- A list of wild type control cell lines is recommended at the end of this file.

## ATCC® No. TCP-1035™ MYC Recommended Culture Conditions

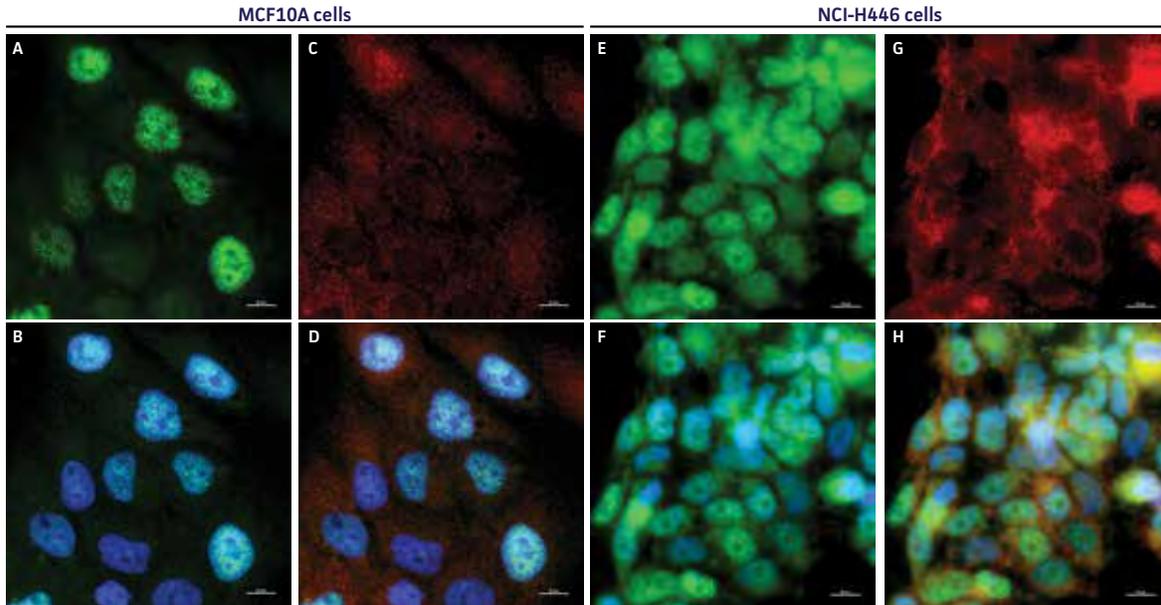
ATCC® No.	Name	Tumor source	Histology	Media	Culture recommendation
CRL-1647™	ST486	ascites	Burkitt's lymphoma	RPMI-1640 + 10% FBS	seeding density: 3.0x10 <sup>5</sup> cells/mL, subculture every 3-4 days, 1:4 to 1:5 split
CRL-1648™	CA46	hematopoietic and lymphoid tissue	Burkitt's lymphoma	RPMI-1640 + 20% FBS	seeding density: 2.0x10 <sup>5</sup> cells/mL, subculture every 2-4 days, 1:8 to 1:12 split
HTB-62™	P3HR-1	ascites	Burkitt's lymphoma	RPMI-1640 + 20% FBS	seeding density: 2.0x10 <sup>5</sup> cells/mL, subculture every 3-4 days, 1:8 to 1:12 split
CRL-5974™	SNU-16	stomach	undifferentiated adenocarcinoma	RPMI-1640 + 10% FBS	seeding density: 2.0-3.0x10 <sup>5</sup> cells/mL, subculture every 3-4 days, 1:3 to 1:5 split
HTB-175™	NCI-H82	lung	small cell carcinoma	RPMI-1640 + 10% FBS	seeding density: 1x10 <sup>4</sup> -5x10 <sup>5</sup> cells/cm <sup>2</sup> , subculture every 2-6 days, 1:2 to 1:8 split
CRL-2081™	MSTO-211H	pleura	mesothelioma	RPMI-1640 + 10% FBS	seeding density: 3.5x10 <sup>4</sup> -6x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-6 days, 1:3 to 1:8 split
HTB-171™	NCI-H446	lung	small cell carcinoma	RPMI-1640 + 10% FBS	seeding density: 3.5x10 <sup>4</sup> -7x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-6 days, 1:3 to 1:9 split

**ATCC® No. TCP-1035™ MYC Recommended Culture Conditions (continued)**

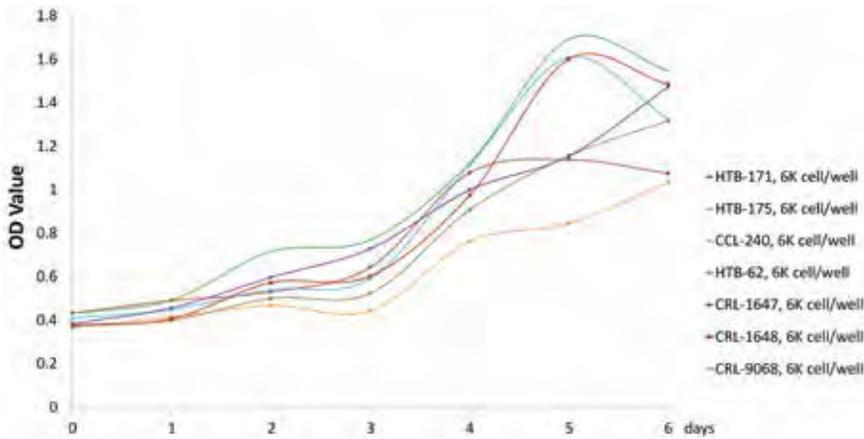
ATCC® No.	Name	Tumor source	Histology	Media	Culture recommendation
CCL-240™	HL-60	peripheral blood	acute myeloid leukaemia	IMDM+20%FBS	seeding density: $1 \times 10^5$ - $5 \times 10^5$ cells/cm <sup>2</sup> , subculture every 2-6 days, 1:2 to 1:8 split
CRL-9068™	NCI-H929	bone marrow	plasma cell myeloma	RPMI-1640 + 0.05 mM 2-mercaptoethanol + 10%FBS	seeding density: $3.0$ - $4.0 \times 10^5$ cells/mL, subculture every 4-7 days, 1:2 to 1:4 split



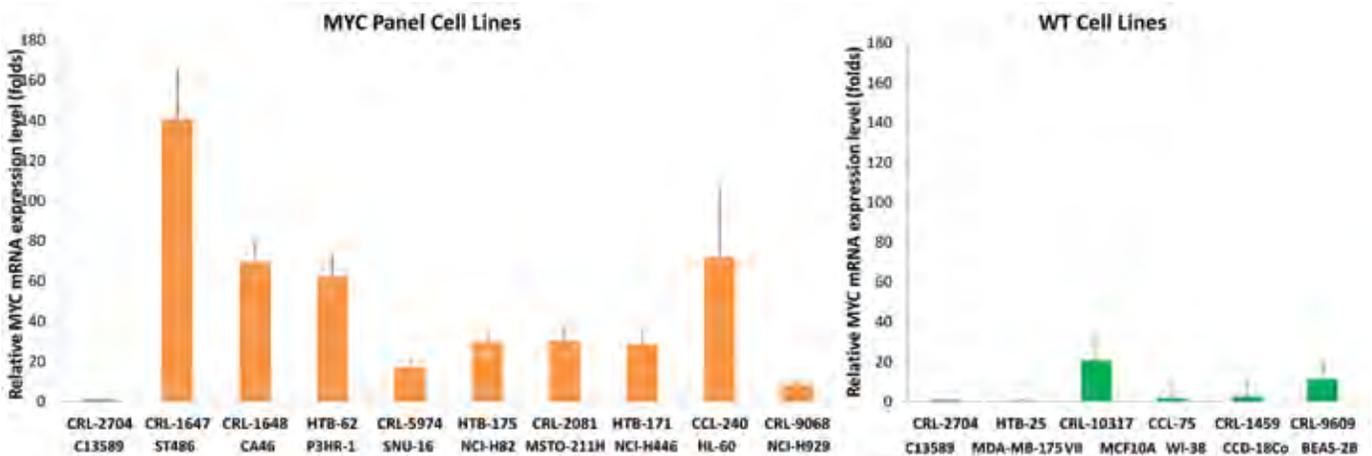
**Figure 1.** Cell morphology of the nine tumor cell lines in the MYC Genetic Alteration Cell Panel. Cells were maintained in ATCC recommended culture condition. Cell morphology was observed under Nikon microscopy, and images of the indicated cell lines were captured by an Olympus digital camera.



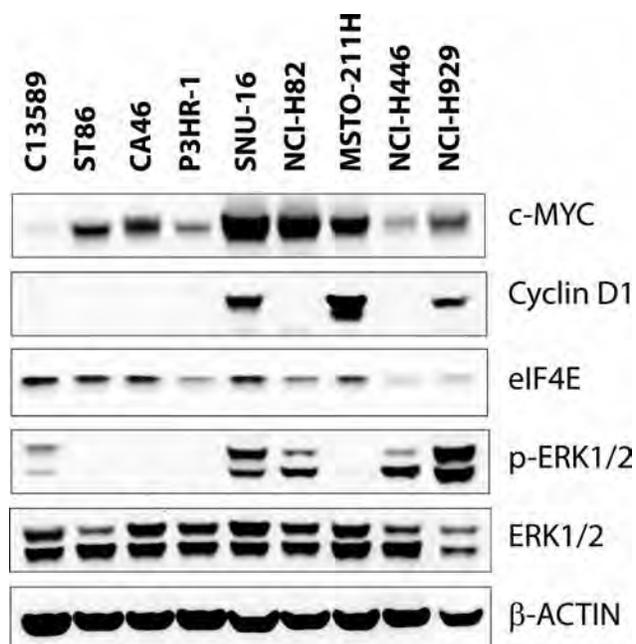
**Figure 2.** Immunofluorescence staining. The recommended MYC WT control cells (A-D) and indicated MYC genetic alteration cells (E-H) were grown on collagen coated coverslips. Cells were fixed with 4% paraformaldehyde. A), B), E) and F): MYC was stained with MYC primary antibody and Alexa Fluor 488 secondary antibody (green). C), D), G) and H): the downstream protein Cyclin D1 was stained with a Cyclin D1 primary antibody and Alexa Fluor 594 secondary antibody (red). Nuclei of the cells were visualized with Hoechst 33342 (blue) in the lower row. Multichannel merged images of indicated protein from above are showed as two-color images (B, F) and as three-color images (D, H).



**Figure 3.** Cell growth kinetics. The indicated genetic alteration cells were cultured in ATCC recommended media, and plated in 96 well plate at 6000 cells/well. The cell growth kinetics were constantly monitored for 6 days by CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega).



**Figure 4.** Real time PCR analysis of mRNA levels. The mRNA expression level of MYC and 36B4 were determined by real time quantitative PCR. Relative MYC mRNA expression of indicated MYC genetic alteration cells were calculated by comparing to WT MCF10A cells after normalization to the housekeeping gene 36B4. The data of recommended WT control cell lines was also provided. Tumor cell lines within the panel were shown as orange, and WT control cell lines were shown as green.



**Figure 5.** Western blotting analysis of endogenous protein expression. The indicated MYC genetic alteration cells and WT C13589 cell line were lysed and processed to extract protein. Western blotting assay was used to examine the total protein level of c-MYC, the protein level of MYC regulated Cyclin D1 and eIF4E, as well as ERK signaling. β-actin protein was examined as a control.

#### Recommended Control Cell Lines

ATCC® No.	Name	Tissue source	Cell Type	Histology
HTB-25™	MDA-MB-175-VII	breast	epithelial	ductal carcinoma
CRL-10317™	MCF 10A	breast	epithelial	normal
CCL-75™	WI-38	lung	fibroblast	normal
CRL-9609™	BEAS-2B	lung	epithelial	normal
CRL-1459™	CCD-18Co	colon	fibroblast	normal
CRL-2704™	C13589	hematopoietic and lymphoid tissue	B lymphoblast	normal

# PI3K GENETIC ALTERATION PANEL (ATCC® NO. TCP-1028™)

The Phosphoinositide 3-kinases (PI3Ks) family has key regulatory roles in many cellular processes, including cell survival, proliferation and differentiation. The importance of the PI3K pathway in human cancers has been established through its binding to oncogenes and activated receptor tyrosine kinases, and the fact that many components of the PI3K pathway are frequently mutated or altered in human cancers. The PI3K catalytic subunit  $\alpha$ -isoform gene (PIK3CA), which encodes p110 $\alpha$ , is frequently mutated in the most common human tumors. These gain-of-function genetic alterations of PIK3CA are exclusively clustered in two hotspot regions in exons 9 and 20, corresponding to the helical and kinase domains of p110 $\alpha$ , such as E545K and H1047R. The PI3K Genetic Alteration Cell Panel (ATCC® No. TCP-1028™) is composed of ten selected human tumor cell lines from various common cancer types that carry hotspot mutations within the PIK3CA gene. The PI3K status of each cell line has been sequenced and validated by ATCC. This panel is useful for PI3K pathway research and PI3K inhibitors anti-cancer drug discovery.

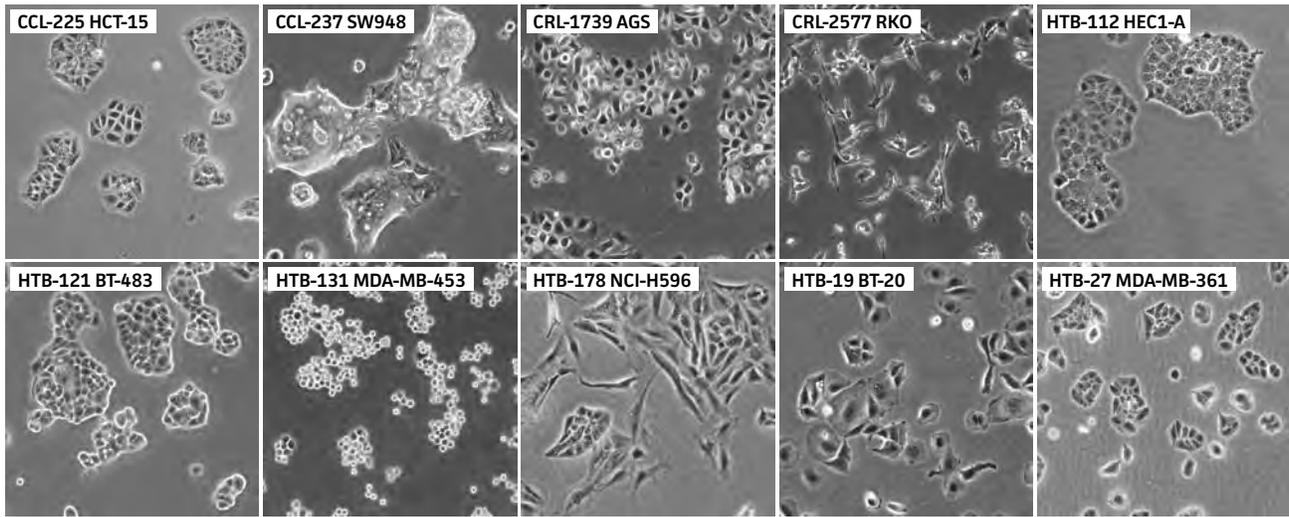
## ATCC® No. TCP-1028™ PI3K Genetic Alteration Cell Panel

ATCC® No.	Name	Gene	DNA Change	Zygosity	Amino acid Change	Tumor source
CCL-225™	HCT-15	PIK3CA	c.1633G>A c.1645G>A	Heterozygous	p.E545K p.D549N	colon large intestine
CCL-237™	SW948	PIK3CA	c.1624G>A	Heterozygous	p.E542K	colon
CRL-1739™	AGS	PIK3CA	c.1634A>C	Heterozygous	p.E545A	stomach
CRL-2577™	RKO	PIK3CA	c.3140A>G	Heterozygous	p.H1047R	colon
HTB-112™	HEC-1-A	PIK3CA	c.3145G>C	Heterozygous	p.G1049R	endometrium
HTB-121™	BT-483	PIK3CA	c.1624G>A	Heterozygous	p.E542K	breast
HTB-131™	MDA-MB-453	PIK3CA	c.3140A>G	Heterozygous	p.H1047R	breast
HTB-178™	NCI-H596	PIK3CA	c.1633G>A	Heterozygous	p.E545K	lung
HTB-19™	BT-20	PIK3CA	c.3140A>G	Heterozygous	p.H1047R	breast
HTB-27™	MDA-MB-361	PIK3CA	c.1633G>A c.1700A>G	Heterozygous	p.E545K p.K567R	breast

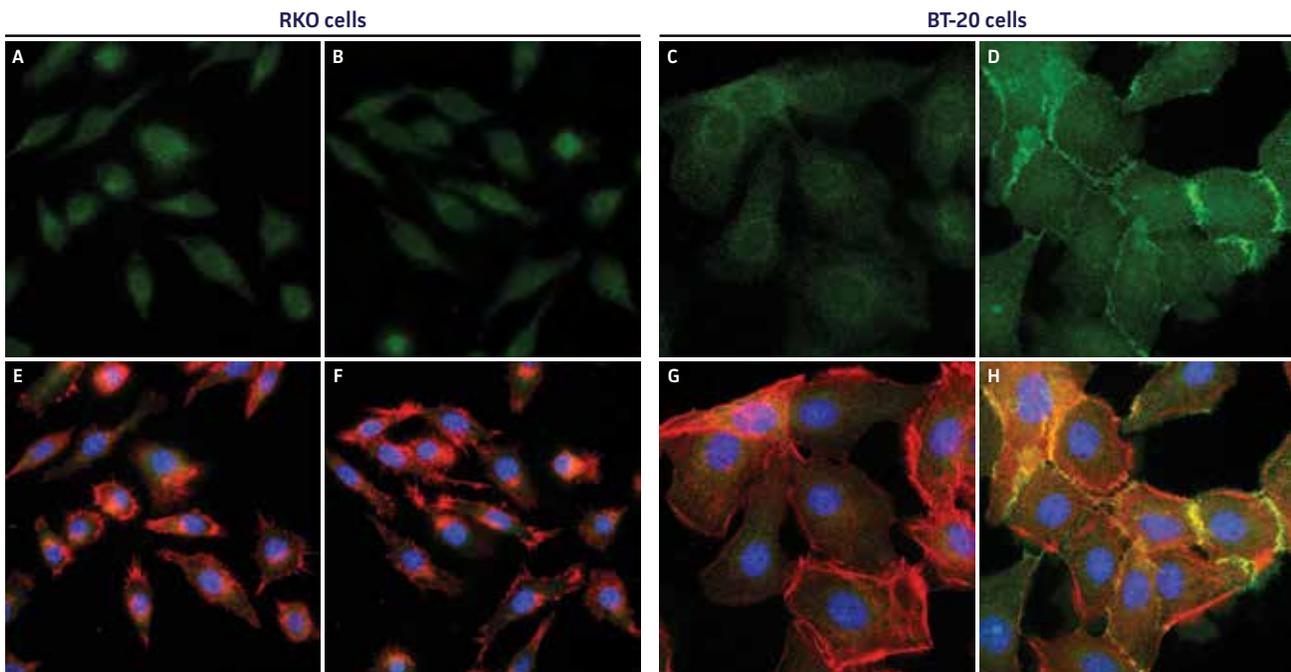
- Somatic mutations analysis was performed by illumina next generation sequencing with greater than 100x coverage. Gene copy number variation analysis was performed by using qBiomarker Copy Number PCR Assay kit from SA Biosciences.
- A list of wild type control cell lines is recommended at the end of this file.

## ATCC® No. TCP-1028™ Recommended Culture Conditions

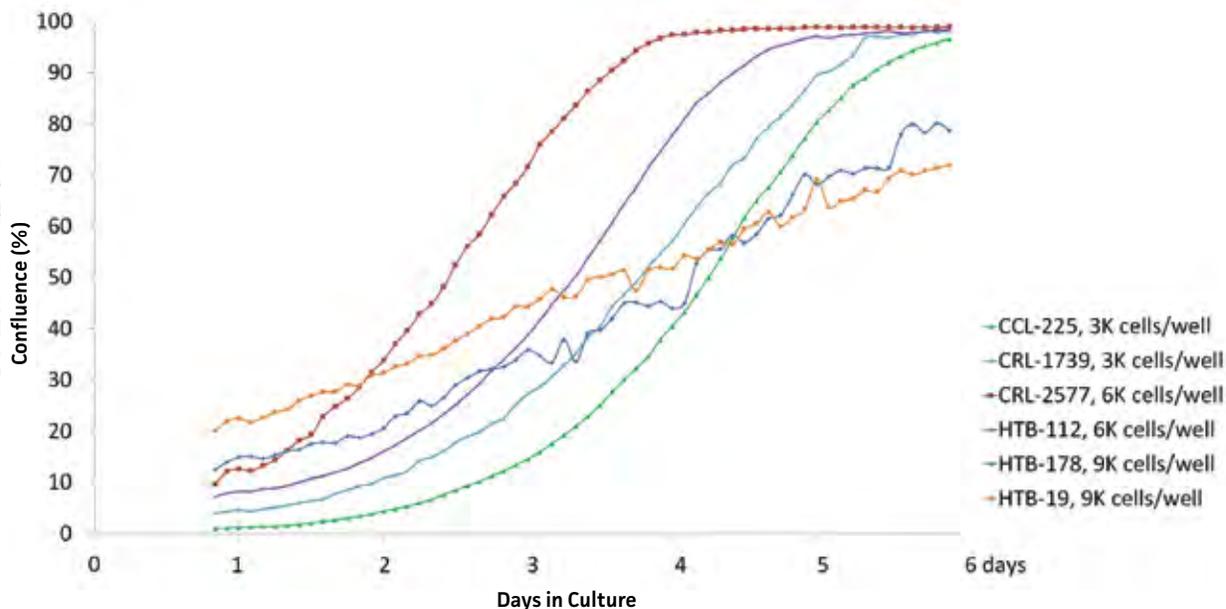
ATCC® No.	Name	Tumor source	Histology	Media	Culture recommendation
CCL-225™	HCT-15	colon	adenocarcinoma	RPMI-1640 + 10% FBS	seeding density: 1.5x10 <sup>4</sup> -2.5x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-4 days, 1:6 to 1:10 split
CCL-237™	SW948	colon	adenocarcinoma	Leibovitz's L-15 + 10% FBS	seeding density: 1.5x10 <sup>5</sup> -2.0x10 <sup>5</sup> cells/cm <sup>2</sup> , subculture every 5-7 days, 1:4 to 1:6 split
CRL-1739™	AGS	stomach	adenocarcinoma	F-12K Medium + 10% FBS	seeding density: 1.0x10 <sup>4</sup> -2.0x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-4 days, 1:5 to 1:8 split
CRL-2577™	RKO	colon	carcinoma	EMEM + 10% FBS	seeding density: 2x10 <sup>4</sup> -4x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-4 days, 1:8 to 1:12 split
HTB-112™	HEC-1-A	endometrium	adenocarcinoma	McCoy's 5A+ 10% FBS	seeding density: 3.0x10 <sup>4</sup> -4.0x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-4 days, 1:4 to 1:6 split
HTB-121™	BT-483	breast	ductal carcinoma	RPMI-1640+ 10 $\mu$ g/mL insulin + 20% FBS	seeding density: 1.5x10 <sup>5</sup> -2.5x10 <sup>5</sup> cells/cm <sup>2</sup> , subculture every 5-7 days, 1:2 to 1:4 split
HTB-131™	MDA-MB-453	breast	carcinoma	Leibovitz's L-15 + 10% FBS	seeding density: 5.0x10 <sup>4</sup> -1.0x10 <sup>5</sup> cells/cm <sup>2</sup> , subculture every 5-7 days, 1:4 to 1:8 split
HTB-178™	NCI-H596	lung	adenosquamous carcinoma	RPMI-1640+ 10% FBS	seeding density: 2.0x10 <sup>4</sup> -4.0x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 5-7 days, 1:2 to 1:4 split
HTB-19™	BT-20	breast	ductal carcinoma	EMEM + 10% FBS	seeding density: 2.0x10 <sup>4</sup> -4.0x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 2-5 days, 1:2 to 1:5 split
HTB-27™	MDA-MB-361	breast	carcinoma	Leibovitz's L-15 + 20% FBS	seeding density: 6x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 4-5 days, 1:5 split



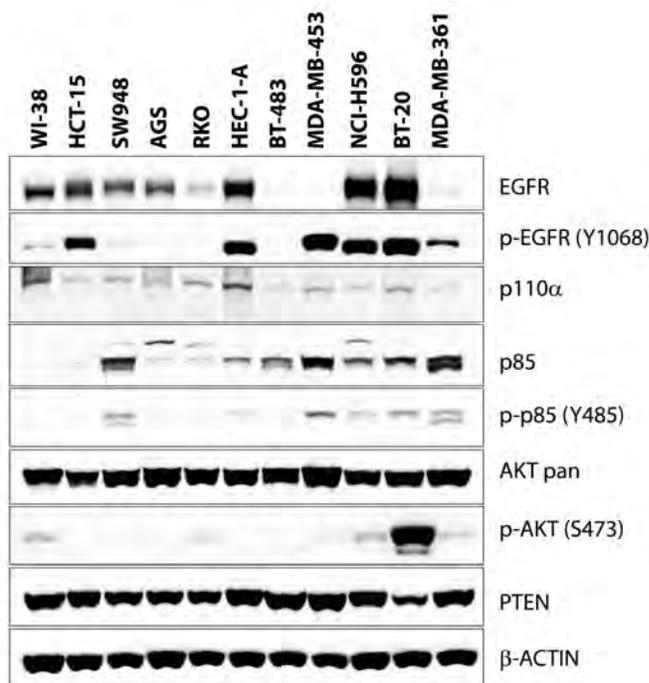
**Figure 1.** Cell morphology of ten tumor cell lines in the PI3K Genetic Alteration Cell Panel. Cells were maintained in ATCC recommended culture condition. Cell morphology was observed under Nikon™ microscopy, and images of the indicated cell lines were captured by Olympus® digital camera.



**Figure 2.** Immunofluorescence staining. The indicated PI3K genetic alternated cells were grown on collagen coated coverslips. Cells were fixed with 4% paraformaldehyde. A) and C), PI3K subunit p110 $\alpha$  was stained with p110 $\alpha$  primary antibody and Alexa Fluor 488 secondary antibody (green). B) and D), phosphor-AKT was stained with phosphor- AKT(S473) primary antibody and Alexa Fluor 488 secondary antibody (green). E) – H) F-actin was visualized with phalloidin Alexa Fluor 594 (red). Nuclei of the cells were visualized with Hoechst 33342 (blue). Multichannel merged images of indicated protein from above, F-actin and nuclei were shown as three-color images.



**Figure 3.** Cell growth kinetics. The indicated genetic alteration panel tumor cell lines were cultured in ATCC recommended media, and plated in 96 well plate at 1000 cells/well - 9000 cells/well seeding density. The cell growth kinetics were constantly monitored for 6 days by using a label-free automated IncuCyte live-cell imaging system (Essen Bioscience).



**Figure 4.** Western blotting analysis of endogenous protein expression. The indicated PI3K genetic alteration cells and WT WI-38 cell line were lysed and processed to extract protein. Western blotting assay was used to examine the protein level and phosphorylation of p85 subunit of PI3K, the protein level of p110 $\alpha$  subunit of PI3K, as well as PI3K upstream and downstream signaling pathways including EGFR, AKT and PTEN.  $\beta$ -actin protein was examined as a control.

### Recommended Control Cell Lines

ATCC® No.	Name	Tissue Source	Cell Type	Histology
HTB-25™	MDA-MB-175-VII	breast	epithelial	ductal carcinoma
CRL-10317™	MCF 10A	breast	epithelial	normal
CCL-75™	WI-38	lung	fibroblast	normal
CRL-9609™	BEAS-2B	lung	epithelial	normal
CRL-1459™	CCD-18Co	colon	fibroblast	normal
CRL-2704™	C13589	hematopoietic and lymphoid	B lymphoblast	normal

# PTEN GENETIC ALTERATION PANEL (ATCC® NO. TCP-1030™)

PTEN is a tumor suppressor that functionally antagonizes PI3K activity by reducing the cellular pool of PtdIns (3,4,5) P3, which is the key second messenger generated by activated class I PI3Ks that drives several downstream signal cascades regulating essential cellular processes. It has been discovered that somatic PTEN gene mutation or deletion occurs frequently in common human tumors. Loss of PTEN leads to hyperplastic proliferation and neoplastic transformation. The PTEN Genetic Alteration Cell Panel (ATCC® No. TCP-1030™) is composed of ten selected human tumor cell lines from various common cancer types that carry PTEN mutations or PTEN deletion. The PTEN status of each cell line has been sequenced and validated by ATCC. This panel is useful for PTEN pathway and PI3K pathway research, as well as anti-cancer drug discovery.

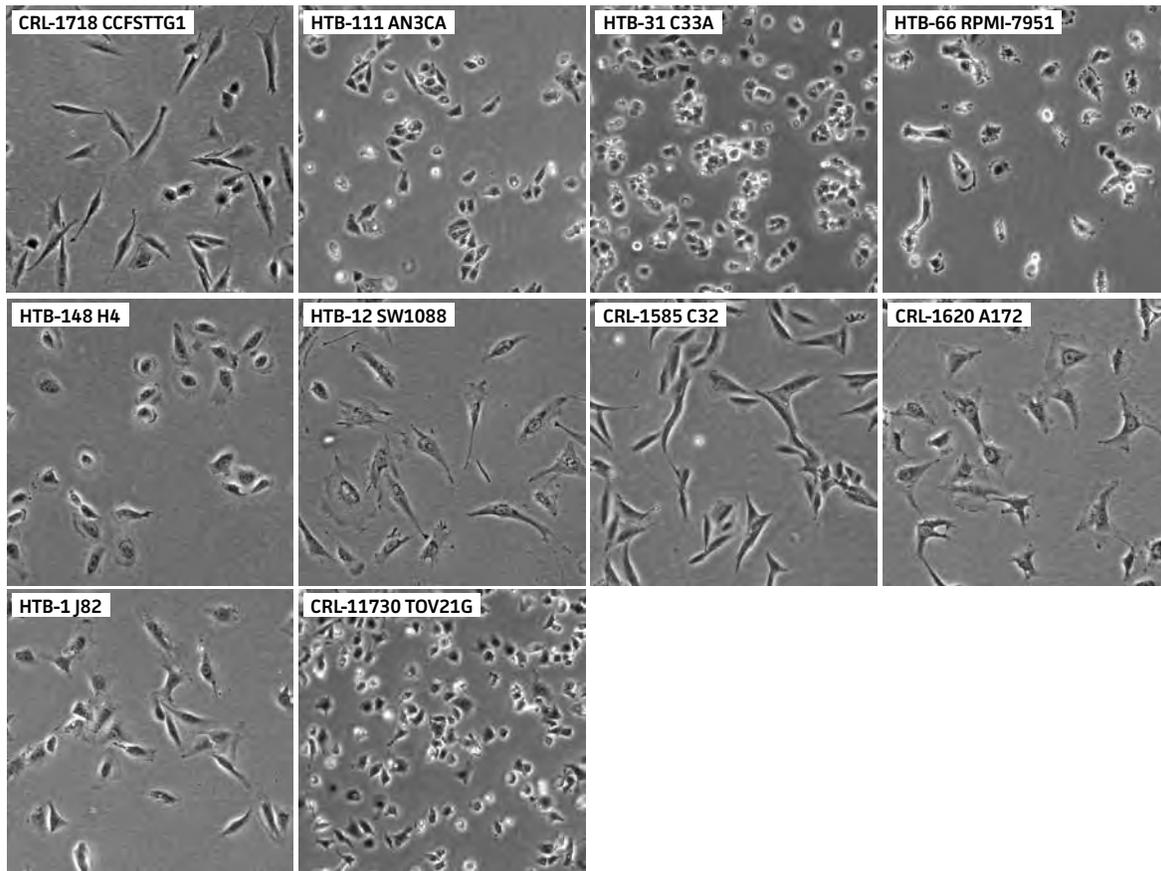
## ATCC® No. TCP-1030™ PTEN Genetic Alteration Cell Panel

ATCC® No.	Name	Gene	DNA Change	Zygoty	Amino acid Change	copy number variation	Tumor source
CRL-1718™	CCF-STTG1	PTEN	c.335T>G	homozygous	p.L112R	–	brain
HTB-111™	AN3 CA	PTEN	c.389_389delG	homozygous	p.R130fs	–	endometrium
HTB-31™	C-33-A	PTEN	c.697C>T	Heterozygous	p.R233*	–	cervix
HTB-66™	RPMI-7951	PTEN	–	–	–	deletion	skin
HTB-148™	H4	PTEN	–	–	–	deletion	brain
HTB-12™	SW 1088	PTEN	–	–	–	deletion	brain
CRL-1585™	C32	PTEN	–	–	–	deletion	skin
CRL-1620™	A172	PTEN	–	–	–	deletion	brain
HTB-1™	J82	PTEN	–	–	–	deletion	urinary bladder
CRL-11730™	TOV-21G	PTEN	–	–	–	–	ovary

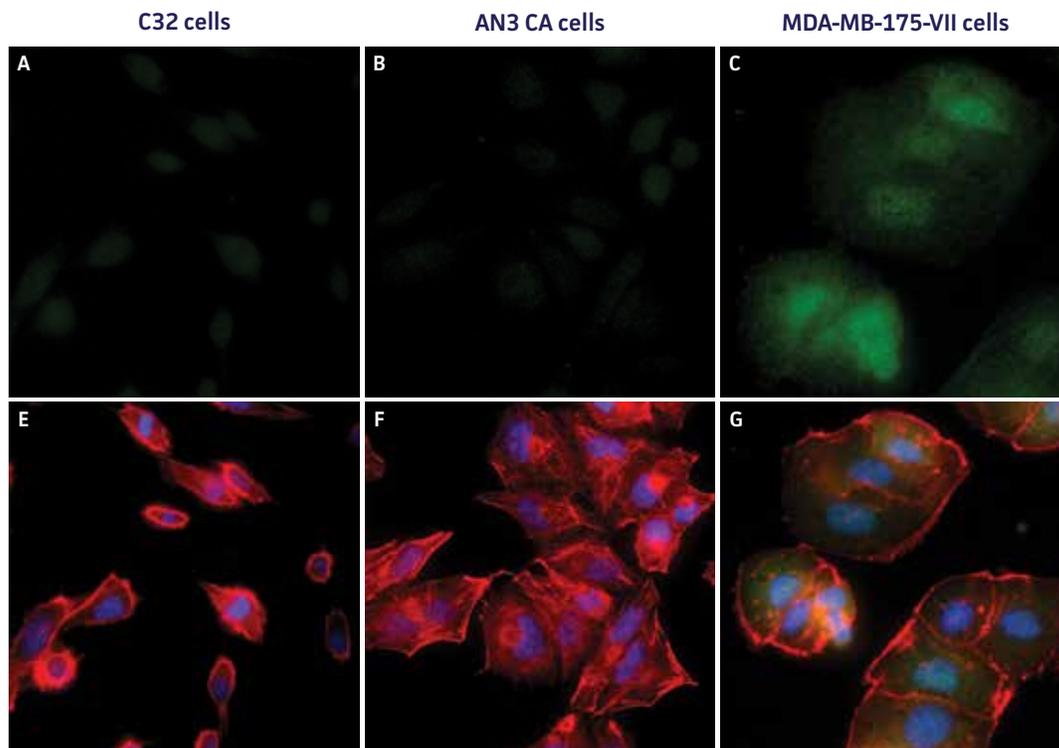
- Somatic mutations analysis was performed by illumina next generation sequencing with greater than 100x coverage. Gene copy number variation analysis was performed by using qBiomarker Copy Number PCR Assay kit from SA Biosciences.
- A list of wild type control cell lines is recommended at the end of this file.

## ATCC® No. TCP-1030™ Recommended Culture Conditions

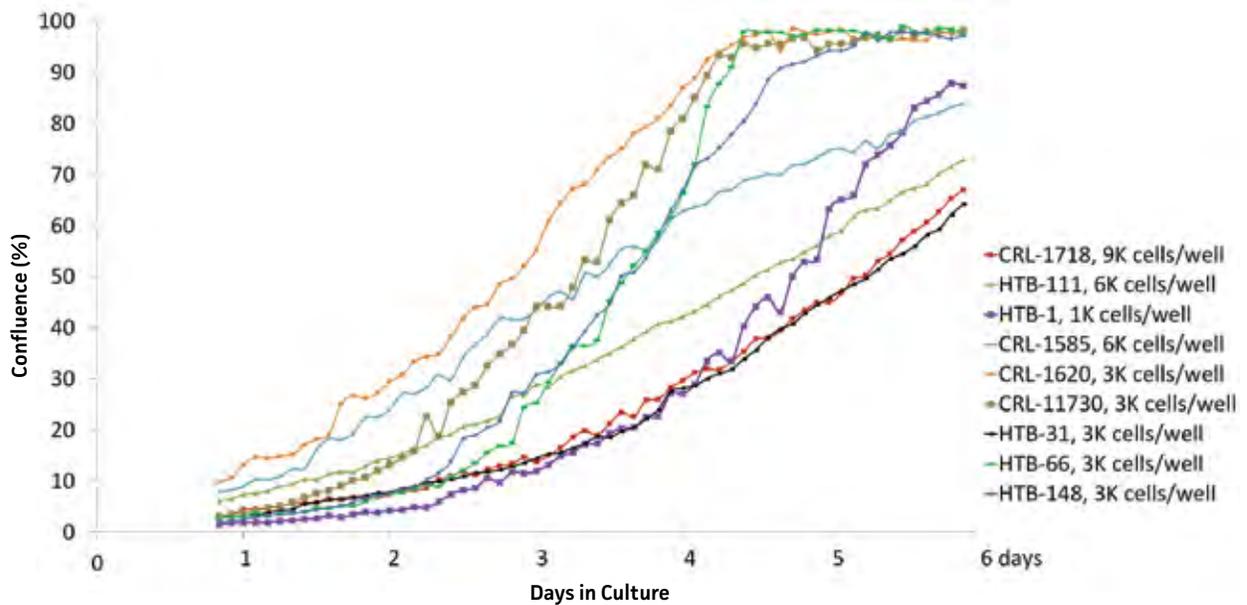
ATCC® No.	Name	Tumor source	Histology	Media	Culture recommendation
CRL-1718™	CCF-STTG1	brain	astrocytoma	RPMI-1640 + 10% FBS	seeding density: 2.0 x 10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 5-6 days, 1:2 to 1:4 split
HTB-111™	AN3 CA	endometrium	adenocarcinoma	EMEM + 10% FBS	seeding density: 3.0x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 7 days, 1:5 to 1:7 split
HTB-31™	C-33-A	cervix	carcinoma	EMEM + 10% FBS	seeding density: 2.0x10 <sup>4</sup> -4.0x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-4 days, 1:8 to 1:10 split
HTB-66™	RPMI-7951	skin	malignant melanoma	EMEM + 10% FBS	seeding density: 2.0x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 5 days, 1:5 to 1:7 split
HTB-148™	H4	brain	neuroglioma	DMEM + 10% FBS	seeding density: 2.0x10 <sup>4</sup> -4.0x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-4 days, 1:8 to 1:12 split
HTB-12™	SW 1088	brain	astrocytoma	Leibovitz's L-15 + 10% FBS	seeding density: 2.0 x 10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 5-6 days, 1:2 to 1:4 split
CRL-1585™	C32	skin	amelanotic melanoma	EMEM + 10% FBS	seeding density: 4.0x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 5 days, 1:8 split
CRL-1620™	A172	brain	glioblastoma	DMEM + 10% FBS	seeding density: 2.5x10 <sup>4</sup> -4.0x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-4 days, 1:8 to 1:10 split
HTB-1™	J82	urinary bladder	transitional cell carcinoma	EMEM + 10% FBS	seeding density: 1.5x10 <sup>4</sup> -2.5x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-4 days, 1:8 to 1:10 split
CRL-11730™	TOV-21G	ovary	clear cell carcinoma	1:1 mixture of MCDB 105 medium + 1.5 g/L NaHCO <sup>3</sup> + Medium 199 + NaHCO <sup>3</sup> 2.2 g/L+15% FBS	seeding density: 1.5x10 <sup>4</sup> -2.5x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-4 days, 1:8 to 1:10 split



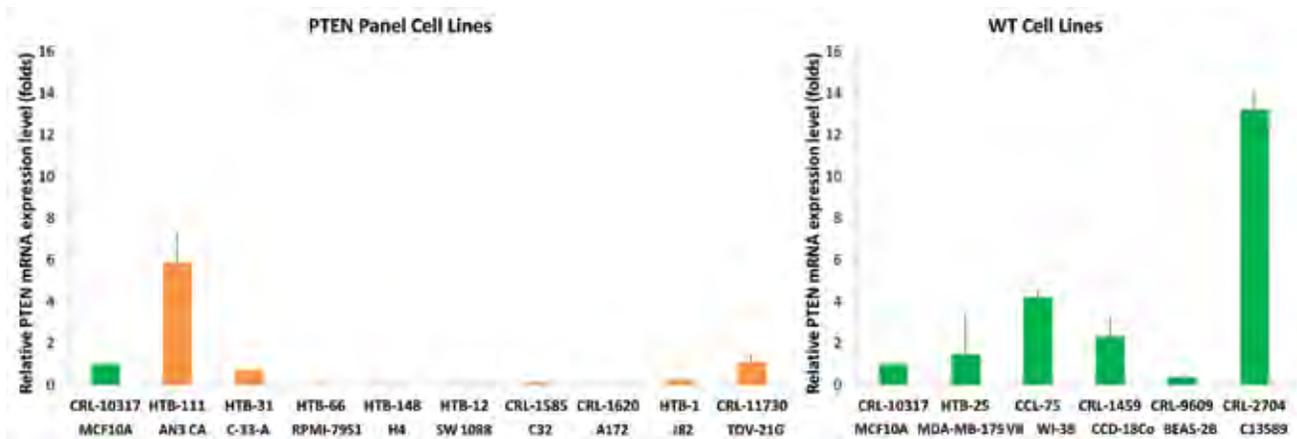
**Figure 1.** Cell morphology of ten tumor cell lines in the PTEN Genetic Alteration Cell Panel. Cells were maintained in ATCC recommended culture condition. Cell morphology was observed under Nikon™ microscopy, and images of the indicated cell lines were captured by Olympus® digital camera.



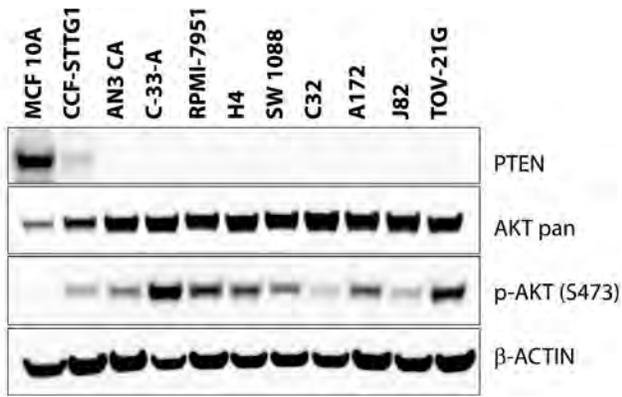
**Figure 2.** Immunofluorescence staining. The indicated PTEN genetic alteration cells and recommended WT control cells were grown on collagen coated coverslips. Cells were fixed with 4% paraformaldehyde. PTEN was stained with PTEN primary antibody and Alexa Fluor 488 secondary antibody (green). F-actin was visualized with phalloidin Alexa Fluor 594 (red). Nuclei of the cells were visualized with Hoechst 33342 (blue). Multichannel merged images of PTEN, F-actin and nuclei were shown as three-color images in the bottom row.



**Figure 3.** Cell growth kinetics. The indicated genetic alteration panel tumor cell lines were cultured in ATCC recommended media, and plated in 96 well plate at 1000 cells/well - 9000 cells/well seeding density. The cell growth kinetics were constantly monitored for 6 days by using a label-free automated IncuCyte live-cell imaging system (Essen Bioscience).



**Figure 4.** Real time PCR analysis of mRNA levels. The mRNA expression level of PTEN and 36B4 were determined by real time quantitative PCR. Relative PTEN mRNA expression of indicated PTEN genetic alteration cells were calculated by comparing to WT MCF10A cells after normalization to the housekeeping gene 36B4. The data of recommended WT control cell lines was also provided. Tumor cell lines within the panel were shown as orange, and WT control cell lines were shown as green.



**Figure 5.** Western blotting analysis of endogenous protein expression. The indicated PTEN genetic alteration cells and WT MCF10A cell line were lysed and processed with to extract protein. Western blotting was used to examine the protein level of PTEN, as well as the downstream signaling of AKT.  $\beta$ -actin protein was examined as a control.

### Recommended Control Cell Lines

ATCC® No.	Name	Tissue Source	Cell Type	Histology
HTB-25™	MDA-MB-175-VII	breast	epithelial	ductal carcinoma
CRL-10317™	MCF 10A	breast	epithelial	normal
CCL-75™	WI-38	lung	fibroblast	normal
CRL-9609™	BEAS-2B	lung	epithelial	normal
CRL-1459™	CCD-18Co	colon	fibroblast	normal
CRL-2704™	C13589	hematopoietic and lymphoid	B lymphoblast	normal

# RAS GENETIC ALTERATION CELL PANEL (ATCC® NO. TCP-1031™)

RAS proteins belong to the super family of small GTPase. The small GTPases are central mediators that act downstream of growth factor receptor signaling and are critical for cell proliferation, survival, and differentiation. RAS can activate several downstream effectors, such as the PI3K-AKT-mTOR pathway and the RAS-RAF-MEK-ERK pathway. More than 20% of human tumors have an activating point mutation in RAS, and this occurs more frequently in KRAS than NRAS. KRAS mutations are particularly common in colon cancer, lung cancer, and pancreatic cancer. In the majority of cases, these mutations are missense mutations in codons 12, 13 and 61. The RAS genetic alteration cell panel (ATCC® No. TCP-1031™) is composed of ten selected human tumor cell lines from various common cancer types that carry KRAS or NRAS hotspot mutations. The KRAS and NRAS status of each cell line has been sequenced and validated by ATCC. This panel is useful for growth factor receptor signaling pathway research, molecular diagnostic biomarker study and anti-cancer drug discovery.

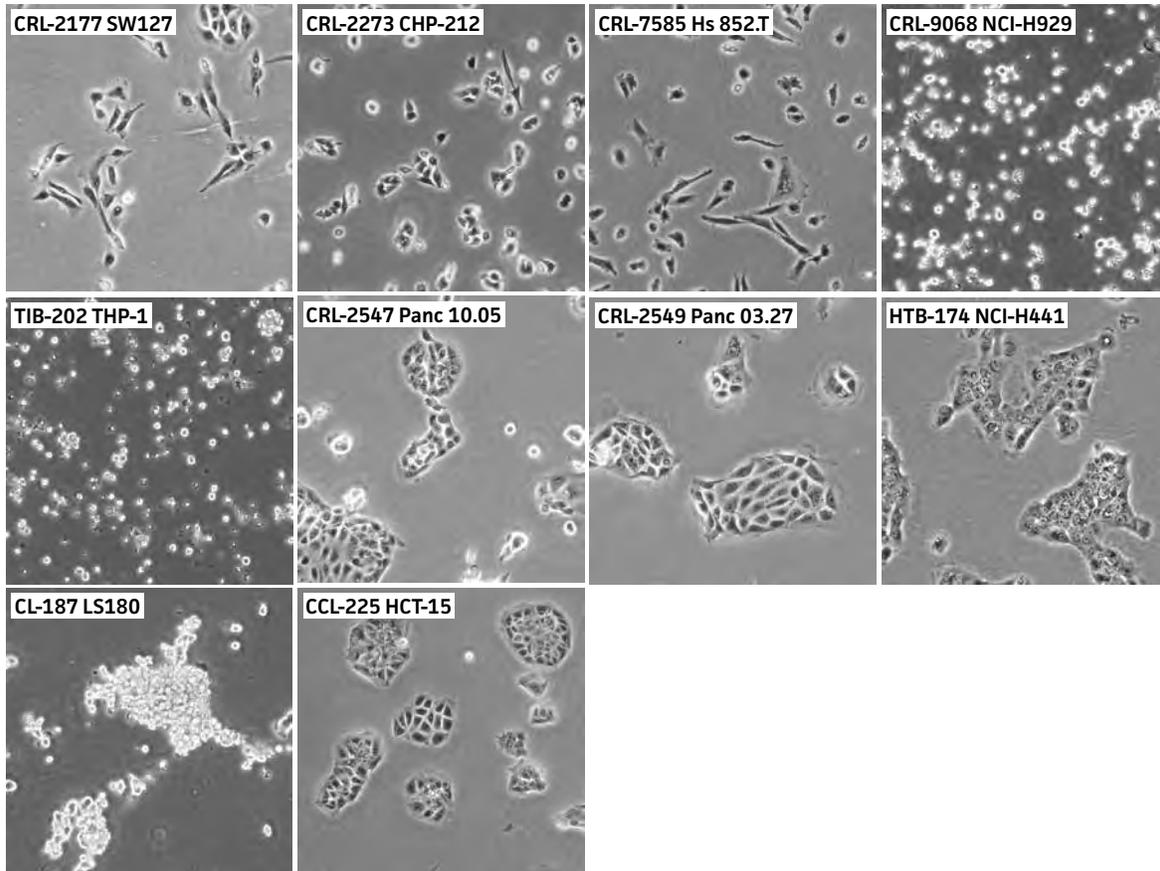
## ATCC® No. TCP-1031™ RAS Genetic Alteration Cell Panel

ATCC® No.	Name	gene	DNA Change	Zygoty	Amino acid Change	Tumor source
CRL-2177™	SW 1271	NRAS	c.182A>G	Homozygous	p.Q61R	lung
CRL-2273™	CHP-212	NRAS	c.181C>A	Heterozygous	p.Q61K	brain
CRL-7585™	Hs 852.T	NRAS	c.35G>T	Heterozygous	p.G12V	skin
CRL-9068™	NCI-H929	NRAS	c.38G>A	Heterozygous	p.G13D	bone marrow
TIB-202™	THP-1	NRAS	c.35G>A	Heterozygous	p.G12D	peripheral blood
CRL-2547™	Panc 10.05	KRAS	c.35G>A	Heterozygous	p.G12D	pancreas
CRL-2549™	Panc 03.27	KRAS	c.35G>T	Heterozygous	p.G12V	pancreas
HTB-174™	NCI-H441	KRAS	c.35G>T	Heterozygous	p.G12V	lung
CL-187™	LS 180	KRAS	c.35G>A	Heterozygous	p.G12D	colon
CCL-225™	HCT-15	KRAS	c.38G>A	Heterozygous	p.G13D	colon

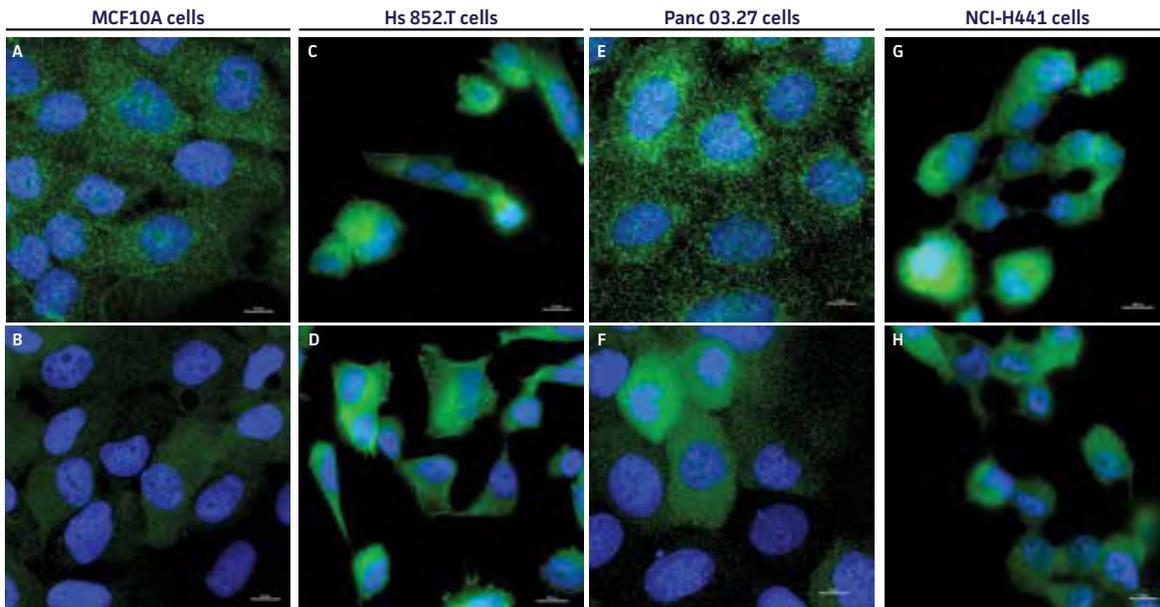
- Somatic mutations analysis was performed by illumina next generation sequencing with greater than 100x coverage. Gene copy number variation analysis was performed by using qBiomarker Copy Number PCR Assay kit from SA Biosciences.
- A list of wild type control cell lines is recommended at the end of this file.

## ATCC® No. TCP-1031™ RAS Recommended Culture Conditions

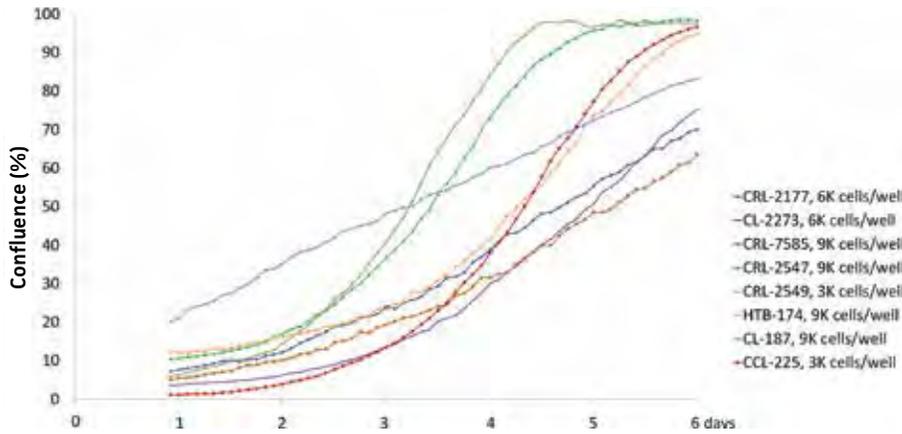
ATCC® No.	Name	Tumor source	Histology	Media	Culture recommendation
CRL-2177™	SW 1271	lung	small cell carcinoma	Leibovitz's L-15+10% FBS	seeding density: 2.0x10 <sup>4</sup> -4x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-5 days, 1:3 to 1:6 split
CRL-2273™	CHP-212	brain	neuroblastoma	1:1 mixture of EMEM and F12 + 10% FBS	seeding density: 2.0x10 <sup>4</sup> -4x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 5-7 days, 1:5 to 1:20 split
CRL-7585™	Hs 852.T	skin	malignant melanoma	DMEM + 10% FBS	seeding density: 2.0x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 4-6 days, 1:3 to 1:5 split
CRL-9068™	NCI-H929	bone marrow	plasma cell myeloma	RPMI-1640 + 0.05 mM 2-mercaptoethanol + 10% FBS	seeding density: 3.0-4.0x10 <sup>5</sup> cells/mL, subculture every 4-7 days, 1:2 to 1:4 split
TIB-202™	THP-1	peripheral blood	acute myeloid leukaemia	RPMI-1640 + 0.05 mM 2-mercaptoethanol + 10% FBS	seeding density: 2.0x10 <sup>5</sup> -4.0x10 <sup>5</sup> viable cells/mL, subculture every 3-5 days, 1:2 to 1:4 split
CRL-2547™	Panc 10.05	pancreas	adenocarcinoma	RPMI-1640+10 Units/mL human recombinant insulin +15% FBS	seeding density: 2.0x10 <sup>4</sup> -4.0x10 <sup>4</sup> viable cells/cm <sup>2</sup> , subculture every 5-7 days, 1:5 to 1:10 split
CRL-2549™	Panc 03.27	pancreas	adenocarcinoma	RPMI-1640+10 Units/mL human recombinant insulin +15% FBS	seeding density: 2.0x10 <sup>4</sup> -4.0x10 <sup>4</sup> viable cells/cm <sup>2</sup> , subculture every 2-4 days, 1:5 to 1:10 split
HTB-174™	NCI-H441	lung	adenocarcinoma	RPMI-1640 + 10% FBS	seeding density: 4.0x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 4 days, 1:3 to 1:5 split
CL-187™	LS 180	colon	adenocarcinoma	EMEM + 10% FBS	seeding density: 1.0x10 <sup>5</sup> cells/cm <sup>2</sup> , subculture every 3-5 days, 1:3 to 1:5 split
CCL-225™	HCT-15	colon	adenocarcinoma	RPMI-1640 + 10% FBS	seeding density: 1.5x10 <sup>4</sup> -2.5x10 <sup>4</sup> cells/cm <sup>2</sup> , subculture every 3-4 days, 1:6 to 1:10 split



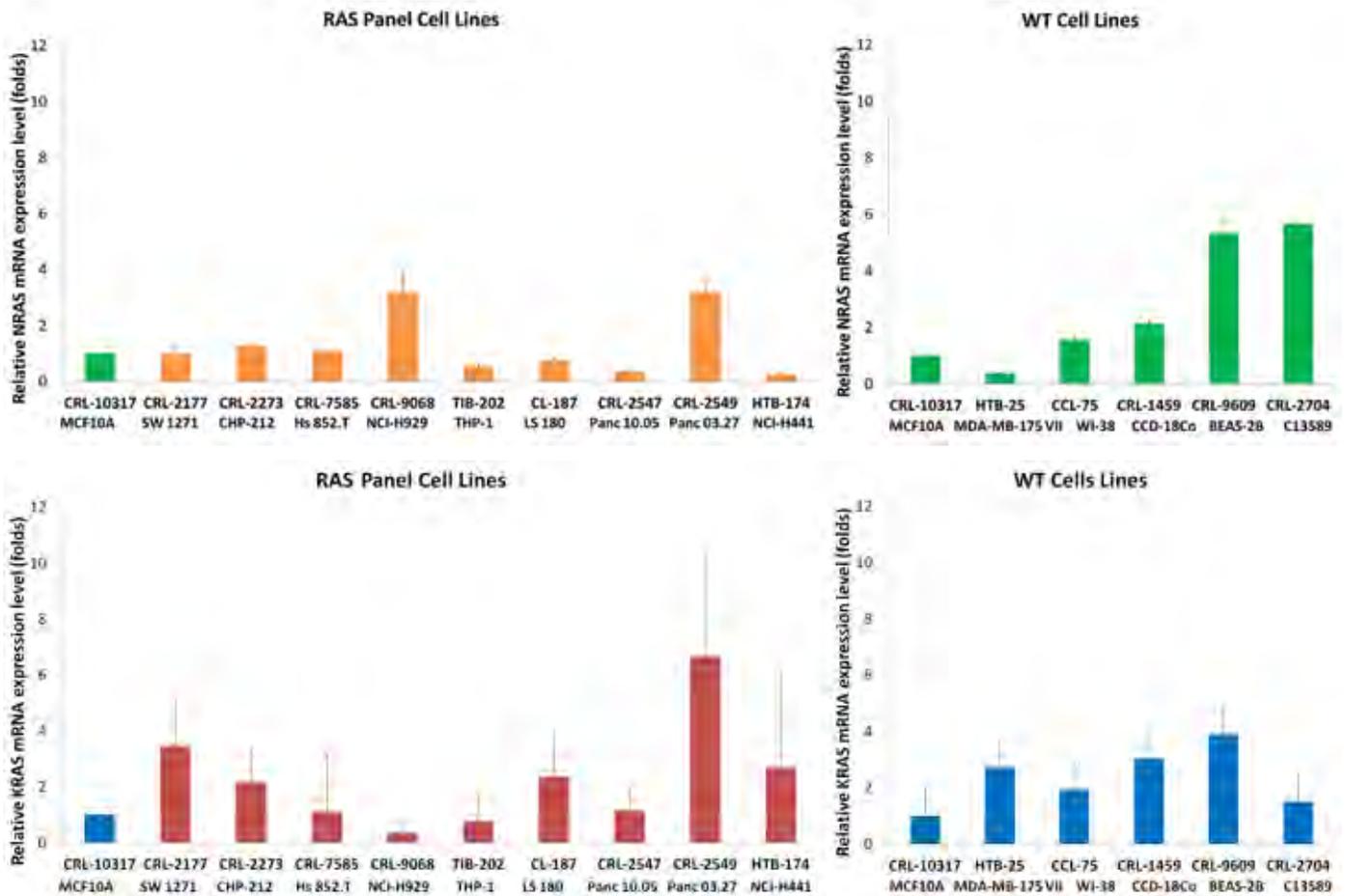
**Figure 1.** Cell morphology of the ten tumor cell lines in the RAS Genetic Alteration Cell Panel. Cells were maintained in ATCC recommended culture condition. Cell morphology was observed under Nikon microscopy, and images of the indicated cell lines were captured by an Olympus digital camera.



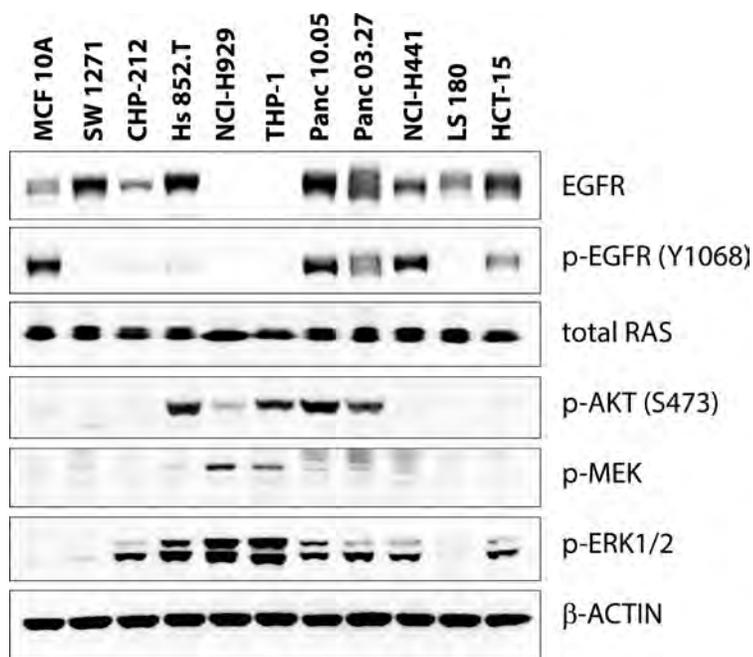
**Figure 2.** Immunofluorescence staining. The recommended RAS WT control cells (A,B) and indicated RAS genetic alteration cells (C-H) were grown on collagen coated coverslips. Cells were fixed with 4% paraformaldehyde. RAS was stained with RAS primary antibody and Alexa Fluor 488 secondary antibody (green) in the upper row (A, C, E, G). The downstream signaling phosphor-ERK1/2 was stained with phosphor-ERK1/2 primary antibody and Alexa Fluor 488 secondary antibody (green) in the lower row (B, D, F, H). Nuclei of the cells were visualized with Hoechst 33342 (blue). Multichannel merged images of indicated protein from.



**Figure 3.** Cell growth kinetics. The indicated genetic alteration panel tumor cell lines were cultured in ATCC recommended media, and plated in 96 well plate at 1000 cells/well - 9000 cells/well seeding density. The cell growth kinetics were constantly monitored for 6 days by using a label-free automated IncuCyte live-cell imaging system (Essen Bioscience).



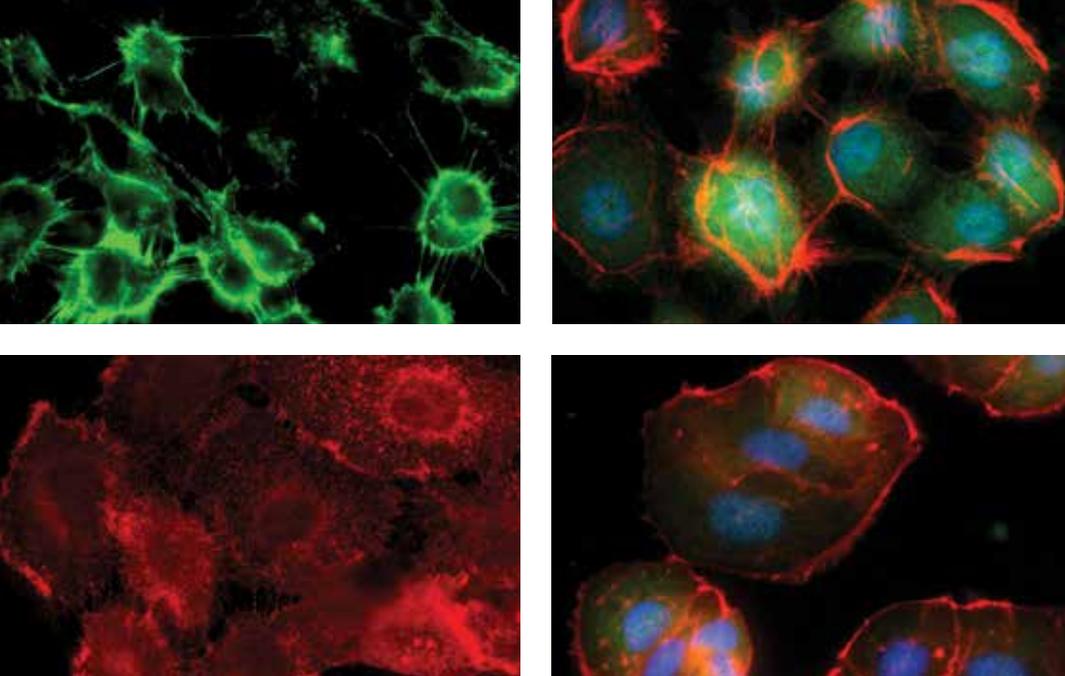
**Figure 4.** Real time PCR analysis of mRNA levels. The mRNA expression level of NRAS, KRAS and 36B4 were determined by real time quantitative PCR. Relative NRAS mRNA expression and KRAS mRNA expression of indicated RAS genetic alteration cells were calculated by comparing to WT MCF10A cells after normalization to the housekeeping gene 36B4. The data of recommended WT control cell lines was also provided. Tumor cell lines within the panel were shown as orange or red, and WT control cell lines were shown as green or blue.



**Figure 5.** Western blotting analysis of endogenous protein expression. The indicated RAS genetic alteration cells and WT MCF10A cell line were lysed and processed to extract protein. Western blotting was used to examine the total protein level of RAS, as well as upstream and downstream signaling pathways including EGFR, AKT, MEK and ERK1/2. β-actin protein was examined as a control.

#### Recommended Control Cell Lines

ATCC® No.	Name	Tissue source	Cell Type	Histology
HTB-25™	MDA-MB-175-VII	breast	epithelial	ductal carcinoma
CRL-10317™	MCF 10A	breast	epithelial	normal
CCL-75™	WI-38	lung	fibroblast	normal
CRL-9609™	BEAS-2B	lung	epithelial	normal
CRL-1459™	CCD-18Co	colon	fibroblast	normal
CRL-2704™	C13589	haematopoietic and lymphoid tissue	B lymphoblast	normal



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*Testing performed for each ATCC cell line was completed on current (2013) distribution material. ATCC provides these data in good faith, but makes no warranty, express or implied, nor assumes any legal liability or responsibility for any purpose for which the data are used.*

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