Quantikine® ELISA

Human Clusterin Immunoassay

Catalog Number DCLU00
SCLU00
PDCLU00

For the quantitative determination of human Clusterin concentrations in cell culture supernates, serum, plasma, saliva, and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Clusterin [also known as Apolipoprotein J, Sulfated Glycoprotein 2 (SGP-2), TRPM-2, and SP-40], is a secreted multifunctional protein that was named for its ability to induce cellular clustering. It binds a wide range of molecules and may function as a chaperone of misfolded extracellular proteins. It also participates in the control of cell proliferation, apoptosis, and carcinogenesis (1, 2). Clusterin is predominantly expressed in adult testis, ovary, adrenal gland, liver, heart, brain, and in many epithelial tissues during embryonic development (3). Human Clusterin is synthesized as a precursor that contains two coiled coil domains, three nuclear localization signals (NLS), and one heparin binding domain (4 - 6). Intracellular cleavages of the precursor remove the signal peptide and generate comparably sized α and β chains which are secreted as an approximately 80 kDa N-glycosylated and disulfide-linked heterodimer (7-9). Mature human Clusterin shares a 77% amino acid sequence identity with mouse and rat Clusterin.

High µg/mL concentrations of Clusterin circulate predominantly as a component of high density lipoprotein particles, and these are internalized and degraded through interactions with LRP-2/Megalin (10, 11). The ability of Clusterin to bind and neutralize non-oxidatively modified LDL reduces cytotoxicity in atherosclerotic plaques (12). The chaperone function of Clusterin helps to reduce the accumulation of β-amyloid fibrils and damage due to amyloid plaques in Alzheimer's disease (13). An alternately spliced 50 kDa isoform of human Clusterin (nCLU) remains intracellular and is neither glycosylated nor cleaved into α and β chains (5, 14). Cellular exposure to ionizing radiation promotes the translocation of nCLU to the nucleus where it interacts with Ku70 and promotes apoptosis (5, 14). This function contrasts with the cytoprotective effect of secreted Clusterin (15). During tumor progression, nCLU is downregulated while the secreted form is upregulated and may be aberrantly glycosylated (14, 16, 17). Increased circulating levels of Clusterin enhance tumor aggressiveness by inhibiting apoptosis and by promoting the epithelial to mesenchymal transition (18-20).

The Quantikine Human Clusterin Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Clusterin in cell culture supernates, serum, plasma, saliva, and urine. It contains NS0-expressed recombinant human Clusterin and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Clusterin showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human Clusterin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Clusterin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Clusterin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for Clusterin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Clusterin bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

PRECAUTIONS

Clusterin is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DCLU00	CATALOG # SCLU00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Clusterin Microplate	893769	1 plate	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with a mouse monoclonal antibody against Clusterin.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Clusterin Standard	893771	1 vial	6 vials	1 µg/vial of recombinant human Clusterin in a buffered protein base with preservatives; lyophilized.	Aliquot and store at ≤ -70 °C for up to 1 month.* Avoid repeated freeze-thaw cycles.
Clusterin Conjugate	893770	1 vial	6 vials	21 mL/vial of monoclonal antibody against Clusterin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-19	895467	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5T	895175	4 vials	24 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative.	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

DCLU00 contains sufficient materials to run an ELISA on one 96 well plate.
SCLU00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDCLU00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human Clusterin Controls (optional; available from R&D Systems).

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

Saliva - Collect saliva directly into a test tube, and centrifuge at 10,000 rpm for 10 minutes. Collect the aqueous layer. Assay immediately or aliquot and store samples at ≤ -80 °C. Avoid repeated freeze-thaw cycles.

Note: *Do not use a saliva collector.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately or aliquot and store at ≤ -80 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 2000-fold dilution. A suggested dilution is 25 µL of sample + 975 µL of Calibrator Diluent RD5T. Complete the 2000-fold dilution by adding 20 µL of the diluted sample to 980 µL of Calibrator Diluent RD5T.

Urine samples require at least a 4-fold dilution. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent RD5T.

Saliva samples require at least a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD5T.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

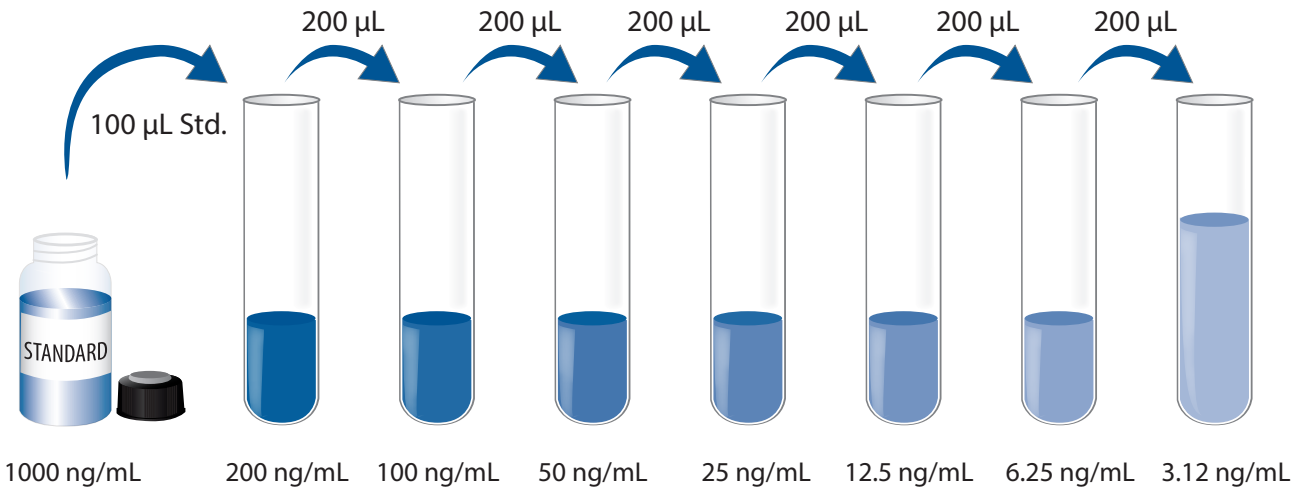
Note: *High concentrations of Clusterin are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Clusterin Standard - Reconstitute the Clusterin Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 1000 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 400 µL of Calibrator Diluent RD5T into the 200 ng/mL tube. Pipette 200 µL of Calibrator Diluent RD5T into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 200 ng/mL standard serves as the high standard. Calibrator Diluent RD5T serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

Note: *High concentrations of Clusterin are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-19 to each well.
4. Add 50 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Clusterin Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

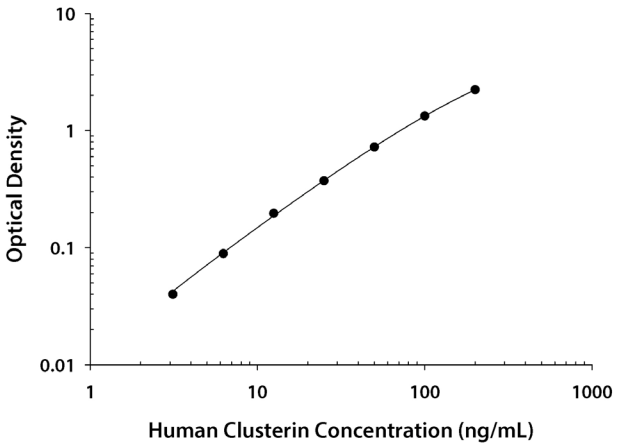
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Clusterin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.006 0.006	0.006	—
3.12	0.045 0.047	0.046	0.040
6.25	0.094 0.096	0.095	0.089
12.5	0.201 0.202	0.202	0.196
25	0.377 0.378	0.378	0.372
50	0.727 0.732	0.730	0.724
100	1.312 1.363	1.338	1.332
200	2.230 2.238	2.234	2.228

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (ng/mL)	19.0	64.3	130	22.1	64.1	136
Standard deviation	0.7	2.4	4.5	1.6	5.4	9.3
CV (%)	3.7	3.7	3.5	7.2	8.4	6.8

RECOVERY

The recovery of Clusterin spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	107	102-110%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of Clusterin were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Saliva (n=4)	Urine (n=4)
1:2	Average % of Expected	103	96	97	99	103	98
	Range (%)	99-110	94-98	93-101	98-99	100-106	95-101
1:4	Average % of Expected	98	94	94	97	102	91
	Range (%)	92-109	92-95	91-97	86-102	97-106	88-92
1:8	Average % of Expected	96	92	91	95	100	89
	Range (%)	89-107	89-95	87-95	85-100	89-107	83-94
1:16	Average % of Expected	92	90	89	92	97	89
	Range (%)	88-97	87-93	86-94	82-99	89-106	83-94

SENSITIVITY

Fifty-seven assays were evaluated and the minimum detectable dose (MDD) of Clusterin ranged from 0.064-1.050 ng/mL. The mean MDD was 0.189 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human Clusterin produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of Clusterin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (µg/mL)	Range (µg/mL)	Standard Deviation (µg/mL)
Serum (n=36)	266	195-378	42.8
Heparin plasma (n=36)	266	190-377	44.6
EDTA plasma (n=36)	261	185-436	52.0

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Saliva (n=17)	334	85.2-624	176
Urine (n=21)	280	85.2-628	171

Cell Culture Supernates:
Human peripheral blood leukocytes (PBLs) were cultured in DMEM supplemented with 5% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of natural Clusterin. All samples were non-detectable.

HepG2 human hepatocellular carcinoma cells were cultured in MEM (NEAA salts) supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for levels of natural Clusterin, and measured 574 ng/mL.

HeLa cervical epithelial carcinoma cells were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for levels of natural Clusterin, and measured 863 ng/mL.

HUVEC human umbilical vein endothelial cells were cultured in EGM-2 media and stimulated with recombinant human IL-1β for 24 hours. An aliquot of the cell culture supernate was removed, assayed for levels of natural Clusterin, and measured 17.3 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Clusterin.

The factors listed below were prepared at 2 µg/mL in Calibrator Diluent RD5T and assayed for cross-reactivity. Preparations of the following factors at 2 µg/mL in a mid-range recombinant human Clusterin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ApoA1
ApoA2
ApoB
ApoB100
ApoC1
ApoC2
ApoD
ApoE
ApoE3
ApoH
ApoM
CLUL-1

Recombinant mouse:

Clusterin

Recombinant rat:

Clusterin

REFERENCES

1. Carver, J.A. *et al.* (2003) IUBMB Life **55**:661.
2. Shannan, B. *et al.* (2006) Cell Death Differ. **13**:12.
3. French, L.E. *et al.* (1993) J. Cell Biol. **122**:1119.
4. Kirszbaum, L. *et al.* (1989) EMBO J. **8**:711.
5. Leskov, K.S. *et al.* (2003) J. Biol. Chem. **278**:11590.
6. Pankhurst, G.J. *et al.* (1998) Biochemistry **37**:4823.
7. Burkey, B.F. *et al.* (1991) J. Lipid. Res. **32**:1039.
8. de Silva, H.V. *et al.* (1990) J. Biol. Chem. **265**:14292.
9. Nilselid, A-M. *et al.* (2006) Neurochem. Int. **48**:718.
10. Jenne, D.E. *et al.* (1991) J. Biol. Chem. **266**:11030.
11. Kounnas, M.Z. *et al.* (1995) J. Biol. Chem. **270**:13070.
12. Schwarz, M. *et al.* (2008) Thromb. Haemost. **100**:110.
13. Nuutinen, T. *et al.* (2009) Brain Res. Rev. **61**:89.
14. Pucci, S. *et al.* (2004) Oncogene **23**:2298.
15. Trougakos, I.P. *et al.* (2004) Cancer Res. **64**:1834.
16. Cochrane, D.R. *et al.* (2007) J. Biol. Chem. **282**:2278.
17. Rodriguez-Pineiro, A.M. *et al.* (2006) Mol. Cell. Proteomics **5**:1647.
18. Lau, S.H. *et al.* (2006) Oncogene **25**:1242.
19. Chou, T-Y. *et al.* (2009) Cell. Signal. **21**:704.
20. Zhang, H. *et al.* (2005) Nat. Cell Biol. **7**:909.