# DuoSet® IC

## Human Total p27/Kip1

Catalog Number DYC2256-2
DYC2256-5
DYC2256E

**For the development of sandwich ELISAs to measure p27 in cell lysates.**

***This package insert must be read in its entirety before using this product.***

**FOR RESEARCH USE ONLY.**
**NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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## PRINCIPLE OF THE ASSAY

This DuoSet® IC ELISA contains the basic components required for the development of sandwich ELISAs to measure p27, also known as Kip1, in cell lysates. An immobilized capture antibody specific for p27 binds both phosphorylated and unphosphorylated p27. After washing away unbound material, a biotinylated detection antibody specific for p27 is used to detect captured protein, utilizing a standard Streptavidin-HRP format.

## MATERIALS PROVIDED

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

| | | | Vials Provided | |
|---|---|---|---|---|
| **Description** | **Part #** | **Storage Conditions** | **Cat. # DYC2256-2** | **Cat. # DYC2256-5** |
| Total p27/Kip1 Capture Antibody | 841669 | 2-8° C | 1 | 2 |
| Total p27/Kip1 Detection Antibody | 841670 | 2-8° C | 1 | 2 |
| Total p27/Kip1 Standard | 841671 | 2-8° C | 3 | 5 |
| Streptavidin-HRP | 890803 | 2-8° C | 1 | 1 |

DYC2256-2 contains sufficient materials to run ELISAs on at least two 96 well plates.*
DYC2256-5 contains sufficient materials to run ELISAs on at least five 96 well plates.*

This kit is also available in an Economy Pack (R&D Systems, Catalog # DYC2256E). Economy Packs contain sufficient materials to run ELISAs on 15 microplates.* Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

*Provided the following conditions are met:

- The reagents are prepared as described in this package insert.
- The assay is run as described in the General ELISA Protocol on page 5.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

## OTHER MATERIALS REQUIRED

- Aprotinin (Sigma # A6279)
- β-Glycerophosphate (Sigma # G6251)
- Leupeptin (Tocris # 1167)
- Pepstatin (Tocris # 1190)
- NP-40 Alternative (EMD/Calbiochem # 492016)
- Sodium Azide ($NaN_3$) (Sigma # S2002)
- Sodium Deoxycholate (Amresco # 0613)
- Sodium Fluoride (NaF) (Sigma # 201154)
- Triton™ X-100 (Sigma # T9284)
- Pipettes and pipette tips
- Deionized or distilled water
- 96 well microplates [Costar EIA Plates (Catalog # 2592 or R&D Systems' Catalog # DY990) are suggested]
- Plate sealers (R&D Systems, Catalog # DY992)
- Squirt bottle, manifold dispenser, or automated microplate washer

## SOLUTIONS REQUIRED

**PBS** - 137 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2HPO_4$, 1.5 mM $KH_2PO_4$, pH 7.2-7.4, 0.2 μm filtered (R&D Systems, Catalog # DY006).

**Wash Buffer** - 0.05% Tween® 20 in PBS, pH 7.2-7.4
(R&D Systems, Catalog # WA126).

**Block Buffer** - 1% BSA,* 0.05% $NaN_3$, in PBS, pH 7.2-7.4.

**IC Diluent #1** - 1% BSA* in PBS, pH 7.2-7.4, 0.2 μm filtered (R&D Systems, Catalog # DY995).

**IC Diluent #15** - 50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40 Alternative, 0.5% sodium deoxycholate, 0.1% SDS.

**Note:** *Approximately 50 mL is required to run the assay on one plate.*

**Lysis Buffer #13**** - 1 mM EDTA, 0.5% Triton X-100, 10 mM NaF, 150 mM NaCl, 20 mM β-Glycerophosphate, 10 μg/mL Leupeptin, 10 μg/mL Pepstatin, 3 μg/mL Aprotinin in PBS, pH 7.2-7.4.

**Substrate Solution** - 1:1 mixture of Color Reagent A ($H_2O_2$) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY999).

**Stop Solution** - 2 N $H_2SO_4$ (R&D Systems, Catalog # DY994).

*The use of R&D Systems Reagent Diluent Concentrate 2 (Catalog # DY995) or Millipore Bovine Serum Albumin, Fraction V, Protease free (Catalog # 82-045) is recommended. All buffers containing BSA must be stored at 2-8° C.

**Sample Diluent Concentrate 1 (5X) (R&D Systems, Catalog # DYC001), prepared as described in the DYC001 insert.

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

**Total p27/Kip1 Capture Antibody** (Part 841669) - Each vial contains 360 μg/mL of goat anti-human p27 antibody when reconstituted with 200 μL of PBS. After reconstitution, store at 2-8° C for up to 30 days or aliquot and store at ≤ -20° C in a manual defrost freezer or at ≤ -70° C for up to 3 months.*

**Total p27/Kip1 Detection Antibody** (Part 841670) - Each vial contains 7.2 μg/mL of biotinylated goat anti-human p27 antibody when reconstituted with 1.0 mL of IC Diluent #1. After reconstitution, store at 2-8° C for up to 30 days or aliquot and store at ≤ -20° C in a manual defrost freezer or at ≤ -70° C for up to 3 months.*

**Total p27/Kip1 Standard** (Part 841671) - Each vial contains 210 ng/mL of recombinant human p27 when reconstituted with 500 μL of IC Diluent #15. **Use within one hour of reconstitution. A fresh standard should be used for each assay.** A seven point standard curve using 2-fold serial dilutions and a high standard of 4000 pg/mL is recommended.

**Streptavidin-HRP** (Part 890803) - 1 mL of Streptavidin conjugated to horseradish-peroxidase. Store at 2-8° C. **DO NOT FREEZE.**

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

## PREPARATION OF SAMPLES

**Cell Lysates** - Rinse cells two times with PBS, making sure to remove any remaining PBS after the second rinse. Solubilize cells at 1 x $10^7$ cells/mL in Lysis Buffer #13 and allow samples to sit on ice for 15 minutes. Assay immediately or store at ≤ -70° C. Before use, centrifuge samples at 2000 x g for 5 minutes and transfer the supernate to a clean test tube. Sample protein concentration may be quantified using a total protein assay. If needed, further dilutions should be made in IC Diluent #15.

## PRECAUTION

The Stop Solution suggested for use with this kit is an acidic solution. Wear protective gloves, clothing, eye, and face protetion. Wash hands thoroughly after handling.

## TECHNICAL HINTS AND LIMITATIONS

- This DuoSet IC ELISA should not be used beyond the expiration date on the kit label.
- Individual results may vary due to differences in technique, plasticware and water sources.
- It is important that the diluents selected for reconstitution and for dilution of the standard reflect the environment of the samples being measured. The diluents suggested in this protocol should be suitable for most cell lysates.
- The type of enzyme and substrate and the concentrations of capture/detection antibodies used can be varied to create an immunoassay with a different sensitivity and dynamic range. A basic understanding of immunoassay development is required for the successful use of these reagents in immunoassays.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Use a fresh reagent reservoir and pipette tips for each step.
- It is recommended that all standards and samples be assayed in duplicate.
- Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay. Buffers containing protein should be made under aseptic conditions and stored at 2-8° C or be prepared fresh daily.

# GENERAL ELISA PROTOCOL

A plate layout is provided to record standards and samples assayed.

### Plate Preparation

1. Dilute the Capture Antibody to the working concentration of 2.0 μg/mL in PBS without carrier protein. Immediately coat a 96 well microplate with 100 μL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of 3 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 for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 μL of Block Buffer to each well. Incubate at room temperature for 1-2 hours.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

### Assay Procedure

1. Add 100 μL of sample or standards in IC Diluent #15 per well. Use IC Diluent #15 as the zero standard. Cover with a plate sealer and incubate 2 hours at room temperature.

   **Note:** *A seven point standard curve using 2-fold serial dilutions and a high standard of 4000 pg/mL is recommended.*
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Dilute the Detection Antibody to a working concentration of 200 ng/mL in IC Diluent #1 immediately before use. Add 100 μL of the diluted Detection Antibody to each well. Cover with a new plate sealer and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Immediately before use, dilute the Streptavidin-HRP to the working concentration specified on the vial label using IC Diluent #1. Add 100 μL of the diluted Streptavidin-HRP to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2 of the Plate Preparation.
7. Add 100 μL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, 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.

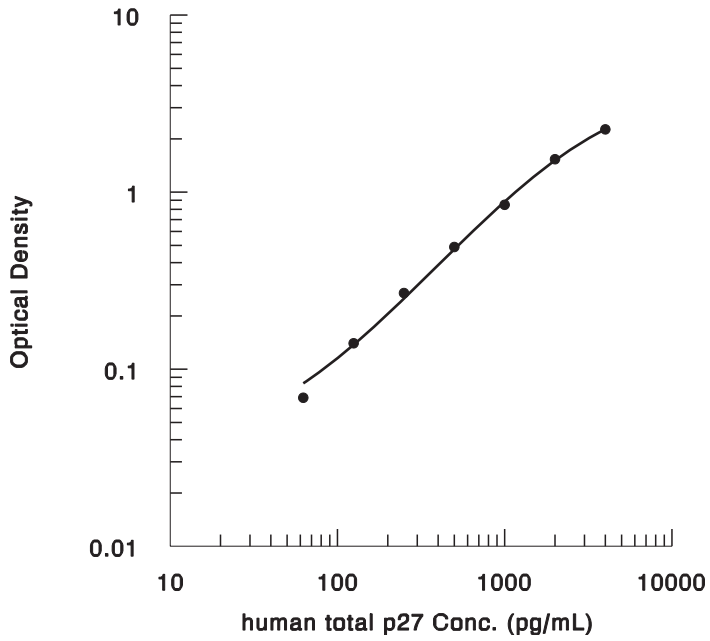
## CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample, then subtract the average zero standard optical density (O.D.).

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 p27 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.

## TYPICAL DATA

A standard curve should be generated for each set of samples assayed. The graph below represents typical data generated when using the Human Total p27/Kip1 DuoSet IC ELISA. The standard curve was calculated using a computer generated 4-PL curve-fit. This standard curve is for demonstration purposes only.




## CALIBRATION

The Human Total p27/Kip1 DuoSet IC ELISA is calibrated against a highly purified *E. coli*-expressed recombinant human p27 produced at R&D Systems. Samples containing natural p27 showed linear dilution parallel to the standard curve obtained using the Total p27/Kip1 Standard. These results indicate that O.D. values from this DuoSet IC ELISA can be used to determine the relative concentration of p27 in natural samples.

## QUANTIFICATION

Amounts of p27, as quantified by the Human Total p27/Kip1 DuoSet IC ELISA, are consistent with the relative amounts of p27 determined by qualitative Western blot analysis.

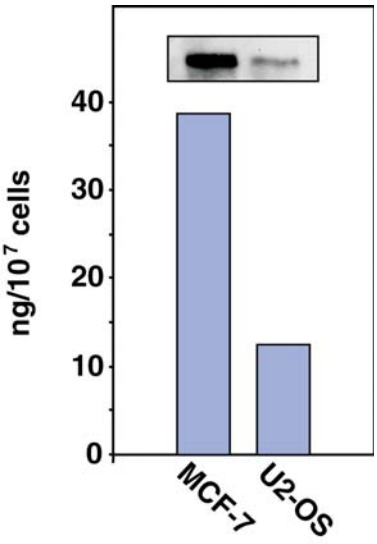
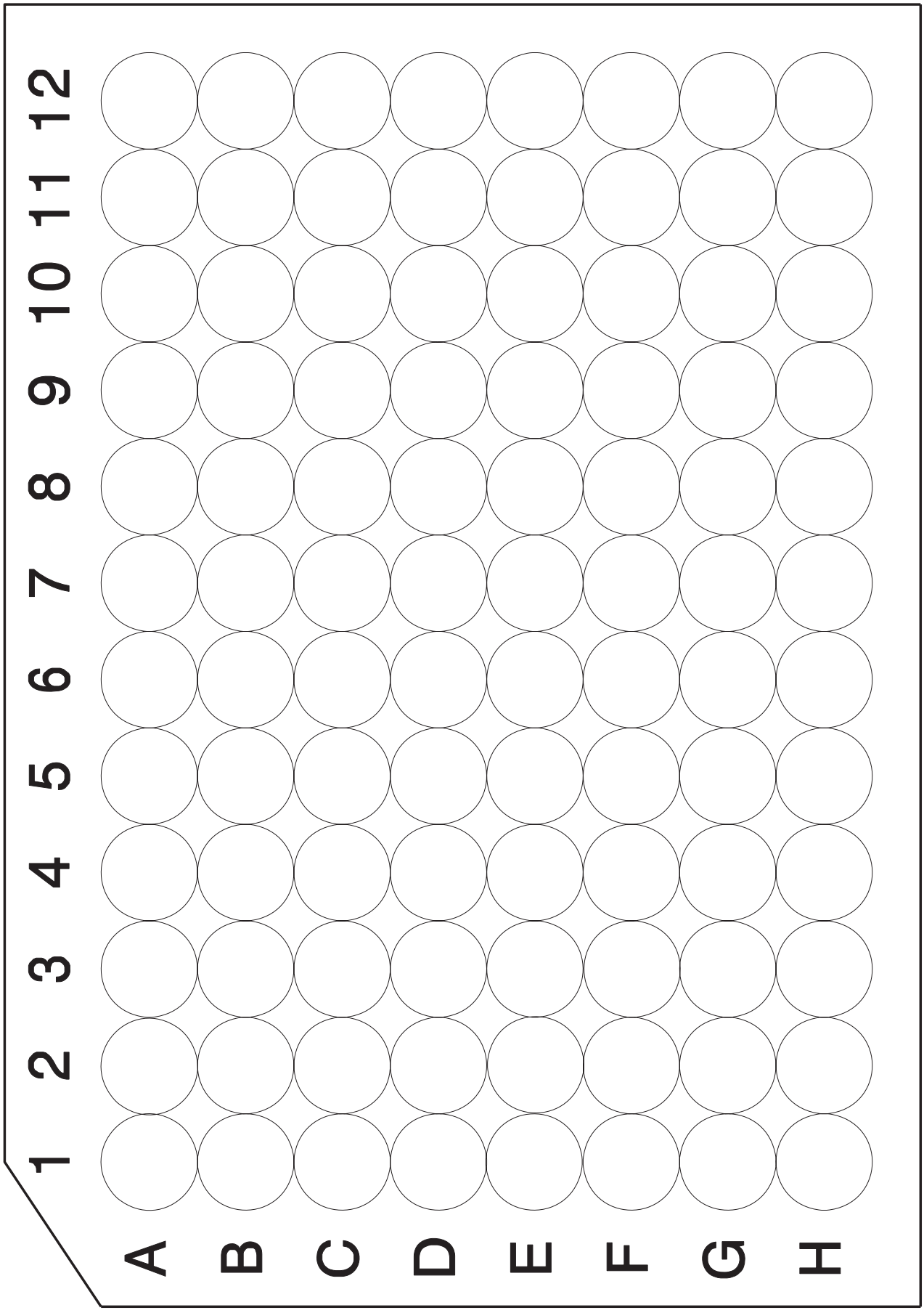



**Figure 1:** Exponentially growing MCF-7 human breast cancer cells and U2-OS human osteosarcoma cells were harvested and cellular lysates were prepared. Total human p27 was quantified with this DuoSet IC ELISA, and the same cellular lysates were immunoblotted (inset) with anti-p27 (R&D Systems, Catalog # AF2256). The DuoSet IC ELISA results correlate well with the relative amounts of total human p27 detected by Western blot.

## PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.



## NOTES

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