Phospho-ATRIP (S224) Cell-Based Colorimetric ELISA Kit

Catalog No. KA1741C

Detection and Quantification of Phospho-ATRIP (S224) Protein Concentration in Cell.

Research Purposes Only. Not Intended for Diagnostic or Clinical Procedures.

CELL-BASED ELISAS FOR MEASURING RELATIVE PROTEIN EXPRESSION AND PHOSPHORYLATION LEVELS

ImmunoWay Biotechnology provides cell-based ELISA kit. Traditional Western blot analysis is time consuming and only semi-quantitative, the Cell-Based Phosphorylation ELISA is an accurate method to determine relative protein levels and degree of phosphorylation among various cell types. Cell-based ELISAs are performed in 96-well microplates, are scalable and conserve cell culture and treatment reagents. Because assay data are measured using a standard ELISA plate reader, the results are readily available for analysis.

Simple, Fast, High Throughput and Cost Efficient

PRODUCT DESCRIPTION

Phospho-ATRIP (S224) Cell-Based Colorimetric ELISA Kit enables the level of Phospho-ATRIP (S224) proteins or degree of different phosphorylation stimulation in different cell types. The relative amount of phospho-specific or total protein is determined using target-specific primary antibody and HRP conjugated secondary antibody detection agent. Following the colorimetric measurement of HRP activity, the crystal violet provided whole cell staining is used for cell number counts. After staining, the results can be analyzed by normalizing the OD values to cell accounts, by which the plating difference can be adjusted.

KIT COMPONENTS

Reagent	Volume	Container Color	Storage
96-Well Cell Culture Clear-Bottom Microplate	-	-	-
Anti-Phospho-ATRIP (S224) antibody (100X)	60 ul	Red Cap	4°C
Anti-ATRIP antibody (100X)	60 ul	Purple Cap	4°C
HRP-Conjugated Anti-Rabbit IgG	5.5 ml	Brown Glass	4°C
Crystal Violet Solution	11 ml	Brown Glass	4°C
Primary Antibody Diluent	11 ml	Colorless Bottle	4°C
Stop Solution	11 ml	Colorless Bottle	4°C
One-Step TMB Substrate	11 ml	Brown Bottle	4°C
1% SDS	22 ml	Colorless Bottle	4°C
Quenching Buffer	22 ml	Colorless Bottle	4°C
Blocking Buffer	50 ml	Colorless Bottle	4°C
Washing Buffer (10X)	50 ml	Colorless Bottle	4°C
Adhesive Plate Seals	4 Seals	-	-

STORAGE AND STABILITY

Upon receipt, the kit should be stored at 4°C. The un-opened kit will be stable for up to 6 months from the

date of shipment if store at 4°C.

ANTIBODY SPECIFICITY

Phospho-ATRIP (S224) antibodies were raised against synthetic phospho-peptide corresponding the residues surrounding the exact phosphorylation site in human proteins molecules and don't cross-react with other sites. Accordingly, total ATRIP antibodies were raised against synthetic peptide corresponding to the residues surrounding either general antigenic domain or the specific site. All the peptides are designed followed the same rules and criteria. The total ATRIP antibody recognizes the corresponding protein regardless of the status of phosphorylation.

CROSS-REACTIVITY

Phospho-ATRIP (S224) Cell-Based Colorimetric ELISA Kit detects phosphorylated proteins and relative total proteins from Human. No cross-reactivity is measured among different phosphorylated sites or species.

BUFFER PREPARATION AND RECOMMENDATION

We provide an excess of buffer components for you in order to perform one 96-well Cell-Based ELISA assay with anti-total-protein antibody.

1X PBS This solution is not provided. 1X PBS is prepared by adding 1 volume of 10 X PBS to 9 volume of distilled water and mixing thoroughly.

Quenching Buffer This solution is provided as ready-to-use. Quenching Buffer is used to inactivate the endogenous peroxidase activity of the seeded cells.

Fixation Buffer This solution is not provided. Fixation buffer is used to fix cell after culture and treatment. It is prepared by adding formaldehyde to 1XPBS and mixing well. 4% formaldehyde is used with adherent cells and 8% formaldehyde is used with suspension cells. The calculation for 4% and 8% formaldehyde solution is based on 37% formaldehyde stock and written Quick Chart for Preparing Buffer.

Washing Buffer Washing Buffer is used throughout the whole Cell-Based ELISA protocol. This buffer is provided as 10X solution. 1X Wash Buffer can be prepared by adding 1 volume of 10X Washing Buffer to 9 volumes of distilled water and mixing well.

Blocking Buffer This buffer is ready-to-use. Blocking Buffer is used to block additional binging sites in each well.

Primary Antibody Diluent This solution is provided as ready-to-use. Use this solution to dilute the provided antibody.

Primary Phospho-specific Antibody This antibody is a rabbit antibody. The phospho-specific antibody recognizes relative molecule phosphorylated at specific site. This supplied antibody is a 100X solution. Make 1:100 dilutions in Primary Antibody Diluent prior to use.

Primary Total-Protein Antibody This antibody is a rabbit antibody. This supplied antibody is a 100X solution. Make 1:100 dilutions in Primary Antibody Diluent prior to use.

HRP-Conjugated Anti-Rabbit IgG Secondary Antibody This solution is provided as ready-to-use. HRP-conjugated anti-rabbit IgG is used as the secondary antibody to detect the target-bound primary antibodies.

1%SDS Solution This solution is provided as ready-to-use. 1% SDS Solution is used in the Crystal Violet procedure to solubilize cells and release the dye for subsequence quantification at 595nm.

Crystal Violet Solution This solution is provided as ready-to-use. Crystal violet is used to determine the relative number of cells in each well. Crystal violet binds to DNA in cell nuclei. OD595 value is proportional to cell numbers. Avoid Contact with skin and clothing.

One-Step TMB Substrate This solution is provided as ready-to-use. Substrate solution should be warmed to room temperature before use. This solution is light sensitive and should be protected from direction exposure to intense light during storage.

Stop Solution This solution is provided as ready-to-use. Prior to each use, transfer the amount of Stop Solution required for the assay into a secondary container. After use, discard any remaining Stop Solution in the secondary container. ***Warning:*** The stop solution is corrosive. Wear personal protection equipment when handling, i.e. labcoat, gloves and eye protection.

ADDITIONAL MATERIALS REQUIRED

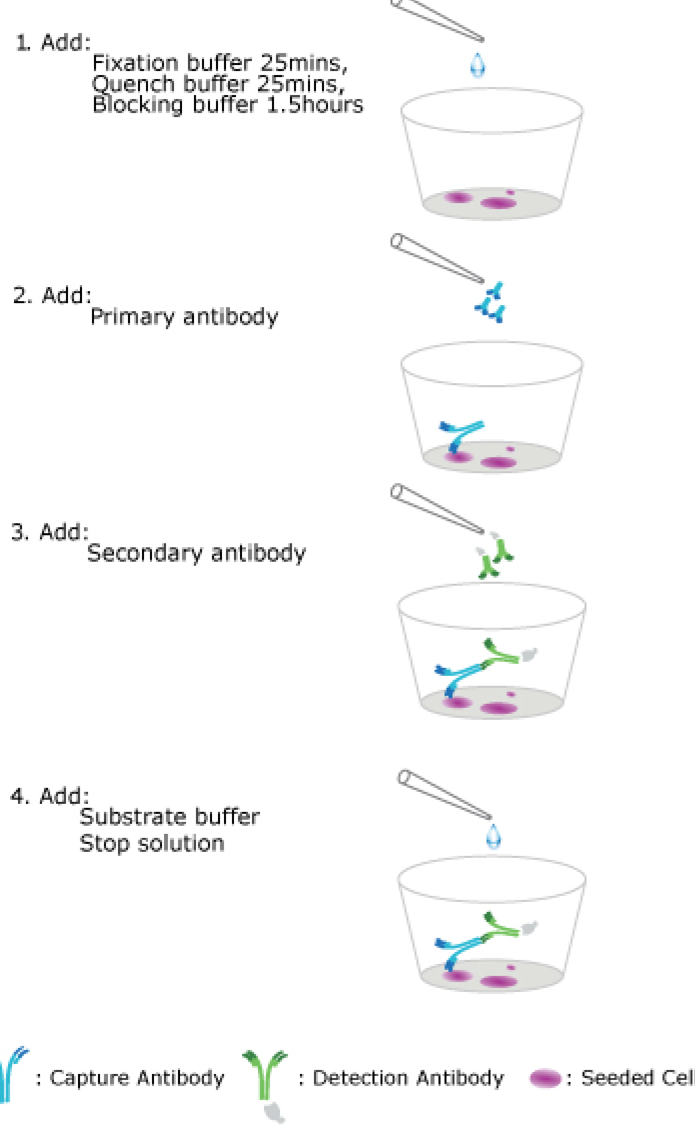
The following materials and equipment are not provided in this kit but are necessary to successfully conduct the experiment:

1. Micropipettes with capability of measuring volumes ranging from 1ul to 1ml
2. Electronic shaker
3. Microplate reader with wavelength of 595nm and 450nm as detection wavelength
4. Squirt bottle, manifold dispenser, multichannel pipette reservoir or automated microplate washer
5. Deionized or sterile water
6. 37% Formaldehyde
7. 10ug/ml poly-L-Lysine
8. 10X PBS

HEALTH AND SAFETY PRECAUTIONS

1. Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin.
2. Fixing Solution contains formaldehyde. Formaldehyde is known to be a highly toxic reagent. Personal protection is strongly recommended while working with this chemical.
3. Stop Solution contains 2N Sulfuric Acid (H_2SO_4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate or strips.
4. Crystal Violent is an intense stain reagent. Avoid contact stain and clothing.

ASSAY OVERVIEW



ASSAY PROTOCOL

Cell Preparation (including cell culture and cell fixation)

1. Seed adherent cells onto the 96-well plates at the different density depending on the size of the cell and desired treatments and incubation time. The cells for testing should be around 75-90% confluent. The plates included in the Kit are sterile and treated for cell culture. For suspension cells and loosely attached cells, coat the 96-well plate with 100ul of 10ug/ml poly-L-Lysine for 30 minutes at 37 °C. Rinse the plate twice with PBS, each rinse for 5 minutes.
2. Culture and treat the cells as desired.
3. Fix cells by removing the cell growth culture medium, following with twice PBS rinse, and a final incubation with 100ul of 4% formaldehyde in PBS for adherent cells and 8% formaldehyde in PBS for suspension cells and loosely attached cells. The incubation can be kept around 25-30 minutes at room temperature. To minimize the vaporization of formaldehyde, the plates are sealed with parafilm. **Note:** Formaldehyde is very volatile, Wear appropriate personal protection equipments (mask, gloves and glasses) when using this chemical.
4. Remove the formaldehyde solution and rinse the cells three times with wash buffer. Each wash step should be minimum five minutes with gentle shaking on the shaker.
5. Add 50ul of PBS into each well of 96-well plate if no ELISA is performed right away and store the plate in the refrigerator for a short of period of time. If long-term storage desired, keep 4% formaldehyde solution and store the plates following the previously mentioned methods.

Cell-Based ELISA: Binding of Primary Antibody and Secondary Antibody

Note: Based on the experiment design, primary antibody incubation can be performed with different anti-total protein antibody. Secondary antibody incubation can be performed with no primary antibody incubation as ELISA negative control.

1. Remove the final Wash buffer, add 100ul of Quench buffer and incubate for 20-25 minutes at room temperature, and seal, cover the plate with parafilm.
2. Remove the Quench buffer and rinse the cells three times for 5 minutes each with 200ul of wash buffer on the shaker.
3. Remove the Wash buffer and add 100ul of Blocking Buffer and incubate for 1-2 hours at room temperature. After blocking, wash the plates three times with Wash buffer for 5 minutes each wash.
4. Dilute the primary antibody with antibody dilution buffer according to the label on the antibody tube.
5. Add 50ul of primary antibody into each well on 96-well plate. Incubate the plate overnight. Seal the plate with parafilm or incubate the plate in a humid-box in refrigerator, and make sure the plate plated at the even level.
6. Remove the primary antibody, wash the wells three times for 5 minutes each with 200ul of Wash buffer with gentle shaking on the shaker.
7. Remove the Wash buffer, add 50ul of secondary antibody diluted in antibody dilution buffer each well, except the empty blank wells. Cover and seal the plates with parafilm, gentle shaking on the shaker for 1-2 hour at the room temperature.
8. During the incubation, prepare Substrate Developing Solution. Transfer the solution to another container

and bring the solution to the room temperature.

Colorimetric Reaction

1. Remove the secondary antibody, wash cells 3-4 times for 5 minutes each time with 200ul of Wash buffer.
2. Add 50ul of Substrate Developing Solution to each well on the 96-well plate.
3. Incubate the plate for 5-25 minutes at room temperature protected from direct light. Closely monitor the blue color development. Don't over develop.
4. Add 50ul of Stop solution. The acidic solution turns blue color into yellow. Make sure that each well develops for the same amount of time.
   Note: Stop solution is corrosive, Wear personal protection equipment while using this solution.
5. Read the absorbance under a micro-plate reader at 450nm with optional reference wavelength of 665nm.

Optional: Crystal Violet Cell Staining

Crystal violet binds to cell nuclei and give absorbance reading at 595nm that is proportional to cell numbers. If normalization OD value from cell numbers is desired, follow the protocol listed below.

1. After finishing reading the absorbance at 450nm, wash the plate twice with 200ul of wash buffer and twice 1X PBS for 5 minutes each. Tap the plates on paper towel to remove the excess liquid. Let plate air dry.
2. Add 50ul of crystal violet solution to each well, incubate on the shaker at room temperature for 30 minutes.
3. Flick the plate to remove crystal violet solution, rinse the plate by filling the wells with tap running water, each wash 5 minutes for 3 times.
4. Add 100ul of 1% SDS into each well and incubate on the shaker at room temperature for 1 hour.
5. Read absorbance at 595nm under microplate reader. You can also dilute the supernatant from each well if absorbance is greater than range.
6. OD450 reading can be normalized by cell number with this formula of OD450/OD595.

TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.immunoway.com or contact us at tech@immunoway.com

ELISA PLATE TEMPLATE

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A												
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C												
D												
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