Version 22-05/25

User's Manual

Keratin 17 ELISA Kit

REF **DEIA-XYA1097**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Keratin 17 Colorimetric Cell-Based ELISA Kit is a convenient, lysate-free, high throughput and sensitive assay kit that can detect Keratin17 protein expression profile in cells. The kit can be used for measuring the relative amounts of Keratin17 in cultured cells as well as screening for the effects that variuos treatments, inhibitors (ie siRNA or chemicals), or activators have on Keratin17.

General Description

The kit is designed for research purposes only and is not intended for diagnostic or clinical procedures. It allows for the detection of Keratin 17 in various cell lines and can be used to measure the effects of different treatments, inhibitors, or activators on Keratin 17 expression.

Principles of Testing

The Colorimetric Cell-Based ELISA Kit enables the detection of target proteins and the effects of stimulation conditions on their expression in different cell lines. The assay is based on an indirect ELISA format where target proteins are captured by target-specific primary antibodies, and HRP-conjugated secondary antibodies bind to the Fc region of the primary antibody. The HRP enzyme catalyzes a colorimetric reaction upon substrate addition, which can be terminated with Stop Solution, allowing for the measurement of absorbance and determination of protein concentration.

The Keratin 17 Cell-Based ELISA Kit is a convenient, lysate-free, high-throughput, and sensitive assay that can monitor target protein Keratin 17 and its expression profile in cells.

Reagents And Materials Provided

1. 96-Well Cell Culture Clear-Bottom Microplate: 1 Plate.
2. 10x TBS: 24 mL (10x), Clear.
3. Quenching Buffer, 24 mL (1x), Clear.
4. Blocking Buffer, 50 mL (1x), Clear.
5. 15x Wash Buffer, 50 mL (10x), Clear.
6. 100x Anti-Keratin 17 Antibody (Rabbit Polyclonal), 60 µL (100x), Purple.
7. 100x Anti-GAPDH Antibody (Mouse Monoclonal), 60 µL (100x), Green.
8. HRP-Conjugated Anti-Rabbit IgG Antibody, 6 mL (1x), Glass.
9. HRP-Conjugated Anti-Mouse IgG Antibody, 6 mL (1x), Glass.
10. Primary Antibody Diluent, 12 mL (1x), Clear.
11. Ready-to-Use Substrate, 12 mL (1x), Brown.
12. Stop Solution, 12 mL (1x), Clear.
13. Crystal Violet Solution, 6 mL (1x), Glass.
14. SDS Solution, 24 mL (1x), Clear.

15. Adhesive Plate Seals, 4 Seals.

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm and/or 595 nm
2. Micropipettes with a measuring range of 1 µl to 1 ml
3. 37% paraformaldehyde stock concentration or 8% paraformaldehyde
4. Deionized or sterile water
5. Squirt bottle, multichannel pipette, and/or automated microplate washer
6. Computer software capable of generating or displaying logarithmic functions
7. Absorbent papers or vacuum aspirator
8. Test tubes or microfuge tubes capable of storing ≥1 ml
9. Orbital shaker, Incubator, and Stimulants
10. Poly-L-Lysine or Poly-D-Lysine for suspension or loosely attaching cells

Storage

2-8°C

Reagent Preparation

Ensure that all supplied solutions are at ambient temperature before use. It is recommended to conduct assays for all controls and samples in duplicate. Sufficient reagents are provided to assay 96 wells; therefore, on the day of the experiment, prepare only the required amount. During incubation steps, use an orbital shaker set at 200 rpm to ensure proper equilibration of solutions in the cell culture plate wells. All other provided solutions are ready to use.

1. Dilute the 10x TBS to 1x TBS by combining 9 volumes of ddH2O and 1 volume of 10x TBS.
2. Dilute the 15x Wash Buffer to 1x Wash Buffer using 14 volumes of ddH2O and 1 volume of 15x Wash Buffer.
3. Prepare the Fixing solution to 4% paraformaldehyde (for adherent cell lines) or 8% paraformaldehyde (for suspension or loosely attaching cell lines). Dilute 37% paraformaldehyde with 1x PBS to desired paraformaldehyde percentage.
4. For suspension or loosely attached cells: Prepare Poly-L-Lysine to working concentration as stated in manual. (Not included, recommended Sigma Cat#

P4832).

If the cell line is known to digest Poly-L-Lysine, Poly-D-Lysine may be an alternative for this step. Follow the manufacturer's instructions if using a different Poly-L-Lysine or Poly-D-Lysine.

5. Prepare a 1x dilution of both 100x primary antibodies anti-Keratin 17 and AntiGAPDH by using the Primary Antibody Diluent at a ratio of 1:100.

Assay Procedure

1. Seed 100 µl of 20,000 adherent cells into each well in a 96-well plate.

Note: Optimal seeding concentrations should be determined by investigator and experimental goals.

For suspension cells and loosely attached cells:

a. Evenly coat plates evenly with 20 uL of Poly-L-Lysine (Sigma Cat# P4832).

b. After 5 minutes, remove the Poly-L-Lysine solution by aspiration and rinse the wells once with tissue culture-grade water.

c. Allow the plate to dry for 2 hours before seeding the cells.

2. Incubate cells overnight or at least 6 hours at 37°C, 5% CO2.
3. Treat the cells as desired.
4. Gently aspirate the cell culture medium and rinse each well twice with 200 µl of 1x TBS.

Note: For loosely attached cells, omit this 1x TBS rinsing step to avoid cell loss.

Instead, proceed directly to the fixing instructions in Step 5 after removing the culture medium.

5. Gently remove the 1x TBS and fix the cells with 100 µl of 8% paraformaldehyde per well. Incubate for 20 minutes at room temperature (RT).

Caution: Paraformaldehyde is volatile. Use appropriate personal protective equipment (PPE) such as gloves, mask, and goggles.

6. Aspirate the solution and add 300 µl of 1x Wash Buffer to each well. Gently shake the plate on an orbital shaker for 2-3 minutes. Repeat this process 3 times.

After the final wash, invert the plate and tap it against clean paper towels to remove any remaining liquid.

Note: After this step, plates can be stored at 4°C for up to a week.

7. Add 100 µl Quenching Buffer to each well and incubate for 20 mins at RT.
8. Repeat the washing process as described in Step 6
9. Add 200 µl of Blocking Buffer and incubate for 1 hour at RT.
10. Repeat the washing process as described in Step 6
11. Add 50 µl of 1x Primary Antibody solutions into each well and incubate overnight at 4°C. If target concentration is known to be high, incubate for 2 hours at RT. Designate 2 wells for negative control by omitting the 1x Primary Antibodies.
12. Repeat the washing process as described in Step 6
13. Add 50 µl of HRP-Conjugated Anti-Rabbit IgG Antibody into the wells incubated with 1x Anti-Keratin 17 Antibody.
14. Add 50 µl of HRP-Conjugated Anti-Mouse IgG Antibody into the wells incubated with 1x Anti-GAPDH Antibody.
15. Incubate the plate for 1.5 hours at RT.
16. Repeat the washing process as described in Step 6
17. Add 50 µl of Ready to use Substrate into each well, cover plate from light, and incubate for 15-20 mins at RT.
18. Add 100 µl of Stop Solution into each well and read at 450 nm.
19. Repeat step 6 and allow plate to air dry for 5 mins at RT.

20. Add 50 µl of Crystal Violet Solution into each well and incubate for 30 mins at RT.

Note: Crystal Violet is an intense stain. Avoid contact with skin and clothing.

21. Dip the plate into a bucket of water in the sink while keeping the water running, and carefully rinse the wells with ddH2O until no more color is visible. Let the plate to dry for 30 mins.
22. Add 100 µl of SDS Solution into each well and incubate for 1 hour at RT.
23. Read absorbance at 595 nm with microplate reader. If absorbance is too high, the solubilized Crystal Violet Solution may be diluted tenfold with ddH2O on a separate 96-well plate.

Calculation

Background subtraction

Average the duplicate or triplicate absorbance readings for each control and sample, subtracting them from the averaged absorbance for wells where primary antibodies have been omitted.

Anti-GAPDH Internal positive control

The OD450 values obtained ensure the assay is functioning, and cell densities between wells should be proportionate to seeding concentration. Stimulation of cells should impact target primary antibody absorbance values, with no effect on the anti-GAPDH absorbance.

Crystal Violet Staining Normalization

The Crystal Violet staining method enables intensity normalization within the same well.

By using the ratio, the OD450 readings obtained can be normalized with the OD595 values. This constitutes a within-well method of analysis.

If the absorbance was too high and a tenfold dilution was required. Multiply all values by 10 prior to normalization.

OD450 anti-Keratin 17/OD595 Crystal Violet.

By doing so, the expression levels of Keratin 17 are adjusted to account for the cell density present in each well.

Stimulated to Non-Stimulated Comparison

After normalization Crystal Violet staining, the resultant proportional values can be used to analyze the effects of stimulants in this assay.

Precautions

• Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin.

• Fixing Solution contains formaldehyde. Formaldehyde is known to be a highly toxic reagent. Personal protection is strongly recommended while working with this chemical.

• Stop Solution contains 2 N Sulfuric Acid (H2SO4) 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.

• Crystal Violet is an intense stain reagent. Avoid contact stain and clothing.

Limitations

• This ELISA kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind.

• Materials included in this kit should NOT be used past the expiration date on the kit label.

• Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.

• Variations in pipetting technique, washing technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in binding affinity of the materials provided.

• The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit.

• Individual results may vary due to differences in technique, plasticware and water sources.