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CELL-BASED FLUOROMETRIC ELISA PROTOCOL - FOR ACETYL-SPECIFIC PROTEIN

Buffer Preparation and Recommendation

We provide an excess of buffer components for you to perform 96-well Cell-Based ELISA assay with each of Acetyl-Specific protein and internal control GAPDH respectively. Required materials but not provided are listed on the data sheet.

Preparation of 1XPBS 1 X PBS is prepared by adding 1 volume of 10XPBS to 9 volume of distilled water and mixing thoroughly.

Preparation of Quenching Buffer Quenching buffer is used to consume and to remove the endogenous peroxidase activity. It is normally fresh prepared by adding hydrogen peroxide to washing buffer. 1% hydrogen peroxide is used for this purpose. The calculation recipe for 1% hydrogen peroxide solution is based on 30% stock solution and written in Quick Chart for Preparing Buffer.

Preparation of Fixation Buffer 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.

Preparation of 1X Washing Buffer Washing buffer is used throughout the whole Cell-Based ELISA protocol and prepared by adding 1 volume of 20X Washing Buffer to 19 volume of distilled water and mixing well.

Blocking Buffer This item is ready-to-use. A small amount of white precipitate may appear if thawed in a warm bath. This doesn't interfere with performance.

Antibody Dilution Buffer This is 2X buffer. Simply dilute with 1XPBS. A small amount of white precipitate may appear if thawed in a warm bath. This doesn't interfere with performance.

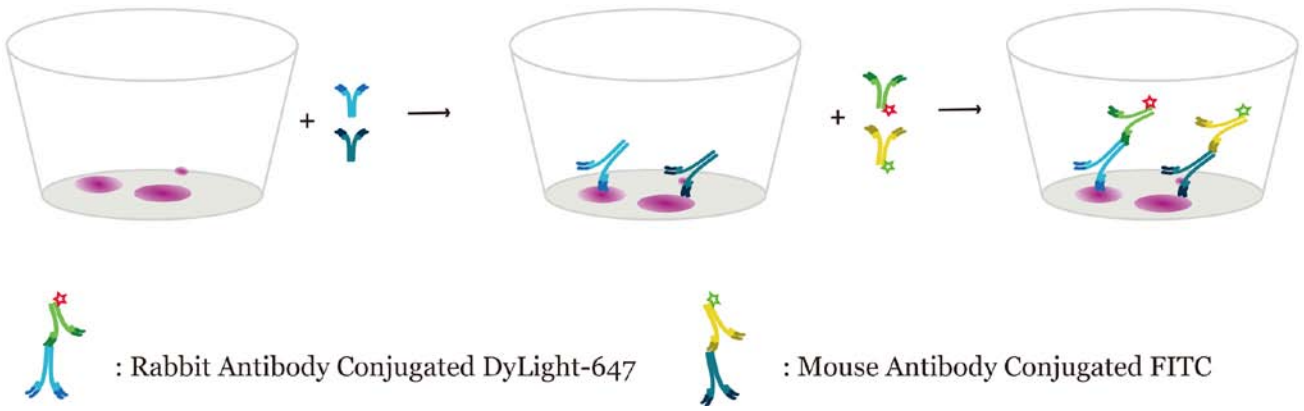
Diluted Acetyl-specific antibody The Acetyl-specific antibody recognizes relative molecule acetylated at specific site. Reconstitute primary Acetyl-specific antibody by dissolving lyophilized antibody into 5ml of antibody diluent. Each well contains 50ul of diluted primary antibody. This provided antibody can be diluted 1:400 in Primary Antibody Dilution Buffer (see Quick Chart for Preparing Buffers).

Diluted internal control anti-GAPDH antibody This GAPDH antibody recognized endogenous total GAPDH

forms of protein. Reconstitute GAPDH antibody by dissolving the lyophilized antibody in 5ml of antibody diluent. Each well contains 50ul of diluted primary antibody.

Diluted DyLight647-Conjugated anti-rabbit IgG Secondary Antibody DyLight 647-conjugated anti-rabbit IgGis used as the secondary antibody to detect bound anti-phophorylation specific primary antibodies. Each well contains 50ul of diluted secondary antibody.

Diluted FITC-conjugated anti-mouse IgG Secondary Antibody FITC-conjugated anti-mouse IgG is used as the secondary antibody to detect bound GAPDH primary antibodies. Each well contains 50ul of diluted secondary antibody.



Adherent Cell Protocol

Cell Preparation of Adherent Cells (including cell culture and cell fixation)

1. Seed 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.
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. The incubation can be kept around 25-30mins 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 Acetyl-specific antibodies and total. 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 20-25mins 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 5mins each wash.
4. Dilute the primary antibody with antibody dilution buffer according to the label on the antibody tube.
5. Add 50ul of each primary antibody (Acetyl-specific antibody and GAPDH antibody) into each relevant well on the 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.

Fluorescence Microplate Reading

1. Remove the secondary antibody, wash cells 3-4 times for 5 minutes each time with 200ul of Wash buffer.
2. Add 50ul of 1 X PBS to each well on the 96-well plate.
3. Read the plate under Excitation and Emission wavelength specific for FITC and DyLight 647.
4. (Optional) Calculate the fluorescence intensity ratio between DyLightvs FITC, compare ratio before and after treatment. Ratio=(Dylight/Exp-Dylight/Con)/(FITC/Exp-FITC/Con)

Suspension Cell Protocol

The protocol for suspension cell based Cell-Based Acetyl-Specific ELISA can be modified from adherent cell based Cell-Based Acetyl-Specific ELISA by seeding and fixing the cells as follows.

1. Pre-treat the 96-well plate with 10ug/ml poly-L-Lysine for 30mins at 37 °C by adding 100ul of 10ug/ml

poly-L-Lysine into each well on 96-well plate. Rinse the plate twice with PBS, each rinse for 5mins.

2. Seed the desired amount of cells for your experimental cells. Grow and treat the cells as your study requires.
3. Remove the cell culture medium and rinse with pre-warmed 1XPBS before formaldehyde fixation. Add 100ul of 8% formaldehyde solution for 20mins fixation incubation at room temperature.
4. Follow the rest of steps written in Adherent Cell Protocol.