Product Manual

WESTERNVIEW™ Dual Detection Kit (Anti-Mouse/Anti-Rabbit)

Catalog No. ENZ-KIT163

For the latest product information, including support documentation, visit us online:
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Product Manual

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

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INTRODUCTION

The WESTERNVIEW™ Dual Detection Kit (Anti-Mouse/Anti-Rabbit) can be used to identify primary antibodies on a Western Blot using immunodetection. The kit contains a complete reagent set, with optimized ready-to-use or ready-to-dilute reagents.

PRECAUTIONS

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1. Kit performance was tested with a variety of antibodies; however it is possible that high levels of interfering substances may cause variation in assay results.

MATERIALS SUPPLIED

1. **Antibody Diluent, 4 x 90 mL**

   Diluent solution for dilution of primary and secondary antibodies.

2. **NBT/BCIP, Ready-to-Use, 100 mL**

   Ready-made solution for chromogenic blotting to be used when developing anti-rabbit secondary.

3. **DAB Chromogen (25X), 4 mL**

   Concentrated chromogen for chromogenic blotting to be used when developing anti-mouse secondary.
   Note: Must be diluted in DAB Substrate Buffer before use.

4. **DAB Substrate Buffer, 100 mL**

   Buffer used to dilute DAB Chromogen (25X).

5. **SignaSure® Buffer Salts, 2 packets**

   Contents used to make SignaSure® Wash Buffer.

6. **Tween®-20, 2 mL**

   Detergent for SignaSure® Wash Buffer.

7. **Secondary Antibody (HRP Anti-Mouse), 2 mL**

   Anti-Mouse secondary antibody for detection of mouse-based primary antibodies in Western Blots. Use DAB Substrate Buffer and Chromogen to develop.

8. **Secondary Antibody (AP Anti-Rabbit), 2 mL**

   Anti-Rabbit secondary antibody for detection of rabbit-based primary antibodies in Western Blots. Use NBT/BCIP solution to develop.

STORAGE

All components of this kit are stable at 4°C until the kit's expiration date.

OTHER MATERIALS NEEDED

1. Deionized or distilled water.
2. Precision pipets for volumes between 5 µL and 1,000 µL.
3. Tubes
4. Incubation trays for membrane
5. Orbital or rocking shaker
6. Primary antibodies validated for use in Western Blot applications

PROCEDURAL NOTES

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Always use gloves when handling reagents.
3. Use clean forceps to handle membranes.
4. Use pure alkaline phosphatase-free water.
5. Do not allow PVDF membranes to dry. If the membrane dries, rinse it first with methanol and then with water before proceeding.
6. Add solutions to the trays slowly to avoid bubbles forming under the membrane.

REAGENT PREPARATION

Secondary Antibody Mixture (Anti-Mouse/Anti-Rabbit)

Prepare Secondary Antibody Mixture (Anti-Mouse/Anti-Rabbit) at 1:100 dilution in Antibody Diluent for each antibody. For example, for 1:100 dilution, add 100 µL Anti-Mouse secondary antibody and 100 µL Anti-Rabbit secondary antibody in 10 mL Antibody Diluent.

***NOTE**: The optimal antibody concentration may need to be experimentally determined.*

DAB Chromogen/Substrate Buffer

Prepare 1X DAB Chromogen by adding 40 µL DAB Chromogen (25X) to 960 µL of DAB Substrate Buffer. Larger membranes may require more solution to completely cover the membrane.

SignaSure® Wash Buffer

Prepare the buffer by dissolving entire contents of 1 packet into 1 liter of distilled or deionized water and add 500 µL Tween®-20 for a concentration of 0.05%. The pH of the solution should be 8.0 ± 0.2. Store reconstituted buffer at 2-8°C.

ASSAY PROCEDURE

1. Primary antibodies (not supplied), can be combined in the same hybridization buffer and incubated simultaneously (typically overnight at 4°C).

   NOTE*: Primary antibodies must be from different host species: rabbit and mouse.*
2. Place the membrane in an appropriate plastic tray and gently wash 3-4 times with enough SignaSure® Wash Buffer to cover the membrane for 10 minutes in orbital or rocking shaker.
3. During the last wash, prepare Secondary Antibody Mixture (Anti-Mouse/Anti-Rabbit) as previously described.
4. Add enough Secondary Antibody solution to completely cover the membrane and incubate for 1 hour at room temperature on orbital or rocking shaker with moderate shaking.
5. Wash 3-4 times for 10 minutes with enough SignaSure® Wash Buffer to cover the membrane in orbital or rocking shaker.
6. To develop the anti-rabbit secondary antibody, incubate the membrane in the dark (cover tray with aluminum foil) with enough NBT/BCIP to cover membrane until purple bands appear (Usually 10 sec to 2.5 minutes). DO NOT SHAKE.
7. Wash 3-5 times in distilled water.
8. To develop the anti-mouse secondary antibody, incubate membrane with 1X DAB Chromogen with enough solution to cover the membrane until orange bands appear (Usually 10 sec to 2.5 minutes). NO NOT SHAKE.

   NOTE: *Always develop the anti-rabbit secondary antibody with NBT/BCIP solution before the anti-mouse secondary antibody with the DAB chromogen solution. Reversal of these steps will result in loss of signal.*
9. Wash 3-5 times in distilled water.
10. For optimal image result, immediately scan the membrane.
11. If necessary, dry the membrane with filter paper or under an infrared lamp and store at 4°C.

TYPICAL RESULTS

The results shown below are for illustration only.

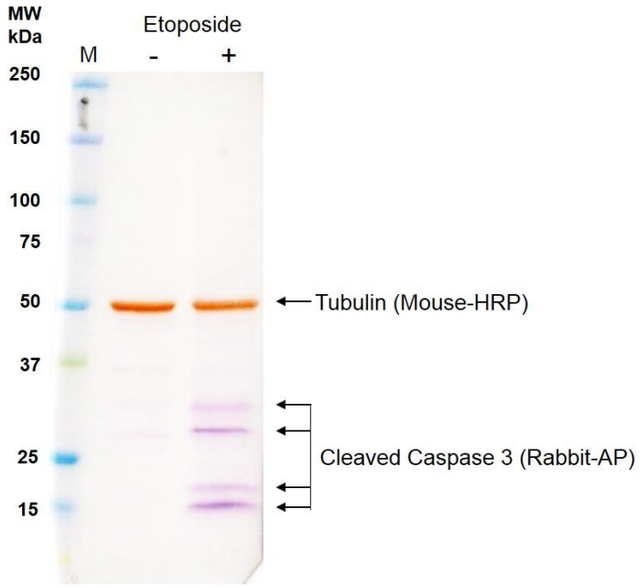

Figure 1. PANC-1 cells (human pancreatic cancer cells) treated with or without etoposide. Loaded a total of 20 µg of cell lysate in each lane. Membrane was incubated with an anti-caspase 3 primary antibody (mouse) and anti-tubulin primary antibody (rabbit).

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