Product Manual

WESTERNVIEW™ Detection Kit (Anti-Mouse)

Catalog No. ENZ-KIT182

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™ Detection Kit (Anti-Mouse) can be used to identify primary mouse 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. Wash Buffer (10X) contains azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up.
2. 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, 100 mL**

   Ready-to-use solution for chromogenic blotting.

3. **Wash Buffer (10X), 2 x 100 mL**

   10X Wash Buffer for washing membrane.

4. **Secondary Antibody (AP Anti-Mouse), 4.5 mL**

   Anti-Mouse secondary antibody conjugated to alkaline phosphatase for detection of mouse-based primary antibodies in Western Blot.

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

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 (AP Anti-Mouse).
Prepare Secondary Antibody (AP Anti-Mouse) at 1:100 dilution in Antibody Diluent. For example, for 1:100 dilution, add 100 µL antibody in 10 mL Antibody Diluent.

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

Wash Buffer (1X)

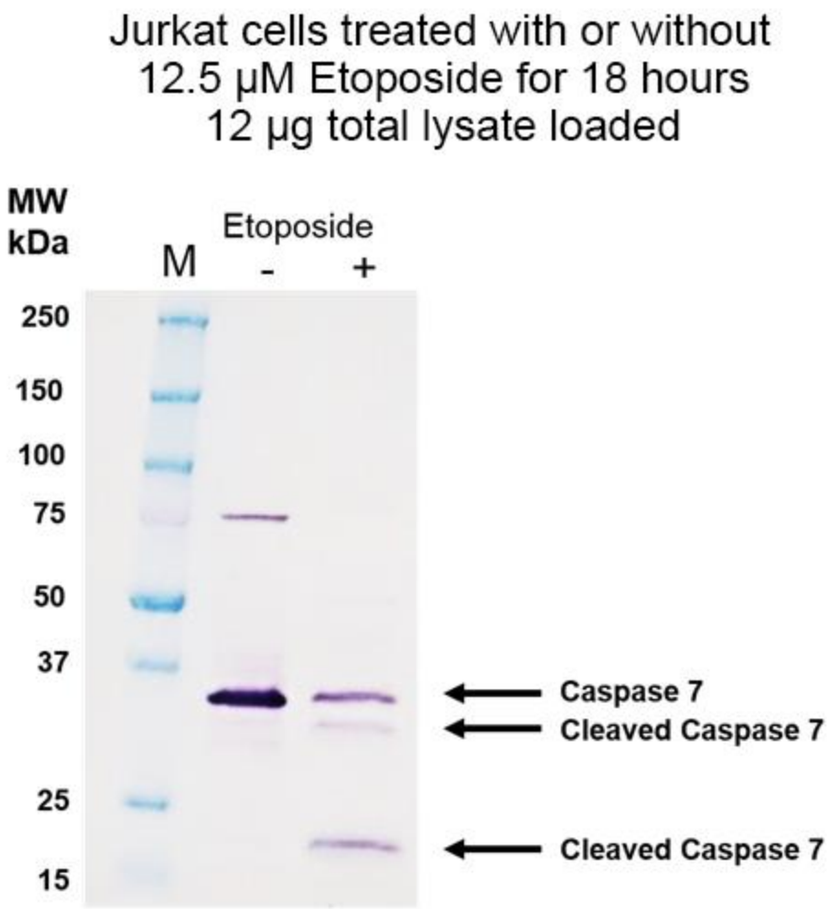
Prepare the 1X Wash Buffer by diluting 10 mL of supplied 10X Wash Buffer with 90 mL of deionized water. This can be stored at room temperature until the kit expiration date, or for 3 months, whichever is earlier.

ASSAY PROCEDURE

1. After incubation with primary antibody, place the membrane in an appropriate plastic tray and gently wash 3-4 times with enough 1X Wash Buffer to cover the membrane for 10 minutes per wash in orbital or rocking shaker.
2. During the last wash, prepare Secondary Antibody (AP Anti-Mouse) as previously described.
3. 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.
4. Wash 3-4 times for 10 minutes per wash with enough 1X Wash buffer to cover the membrane in orbital or rocking shaker.
5. Incubate the membrane in the dark (cover tray with aluminum foil) with enough NBT/BCIP to cover membrane until purple bands appear on the membrane (Usually 10 sec to 2.5 minutes). DO NOT SHAKE.
6. Wash 3-5 times in distilled water.
7. For optimal image result, scan the membrane after step 6.
8. 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.



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