

ORDERING INFORMATION

Catalog Number: AF3297

Lot Number: WKS01

Size: 100 µg

Formulation: 0.2 µm filtered solution in PBS with 5% trehalose

Storage: -20° C

Reconstitution: sterile PBS and 0.02% NaN₃

Specificity: human XPE/DDB2

Immunogen: *E. coli*-derived recombinant human XPE/DDB2 (aa 1-427)

Ig Type: affinity-purified goat IgG

Application: Western blot

Background

Xeroderma Pigmentosum complementation group E (XPE) patients are defective in a heterodimer of damage-specific DNA-binding activity (DDB1/DDB2). DDB2 is a 48 kDa protein upregulated in cells exposed to ultraviolet light. XP patients exhibit hypersensitivity to ultraviolet light and have a heightened incidence of skin cancer.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived, recombinant human XPE/DDB2 (amino acids 1 - 427; GenBank Accession # NM_000107). Polyclonal antibody was affinity-purified on a column derivatized with rhXPE/DDB2, and further purified by isolating the IgG fraction.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute the antibody with 100 µL of sterile PBS containing 0.02% NaN₃.

Storage

The reconstituted antibody should be aliquoted and stored at -20° C in a manual defrost freezer until use. **Avoid repeated freeze/thaw cycles.**

Specificity

The antibody detects human XPE/DDB2.

Application

Western blot - An antibody concentration of 0.5 µg/mL is recommended.

Protocol for Immunoblotting:

Blotting Buffer

25 mM Tris, pH 7.5
0.15 M NaCl
0.05% Tween 20

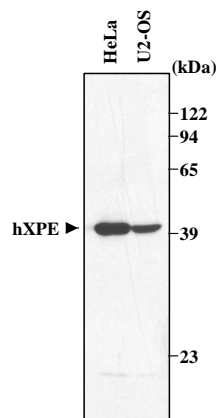
Blocking Solution

5% nonfat dry milk
in blotting buffer
pH to 7.5

1. Transfer the electrophoresed proteins onto a PVDF membrane and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 2 - 8° C in Blocking Solution containing 0.5 µg/mL goat anti-hXPE/DDB2 antibody.
3. Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Blocking Solution containing a 1:2,000 dilution of HRP-conjugated donkey anti-goat Ig (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with Western Glo Chemiluminescent detection reagents (R&D Systems, Catalog # AR004) or equivalent.

Cell lysates for western blotting: To prepare total cell lysates, solubilize cells in 2X SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) and sonicate with a probe sonicator using 3-4 bursts of 5-10 seconds each. Heat extracts in a boiling water bath for 5 minutes and load onto polyacrylamide gels. Samples may be diluted with 1X SDS sample buffer to the desired concentration.

Optimal dilutions should be determined by the individual laboratory.



Extracts from exponentially growing HeLa and U2-OS cells were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 0.5 µg/mL goat anti-hXPE/DDB2 antibody.

