

3050 Spruce Street Saint Louis, Missouri 63103 USA Telephone 800-325-5832 • (314) 771-5765 Fax (314) 286-7828 email: techserv@sial.com sigma-aldrich.com

ProductInformation

Anti-XPB

produced in rabbit, affinity isolated antibody

Catalog Number X0879

Product Description

Anti-XPB is developed in rabbit using as immunogen a synthetic peptide corresponding to amino acids 765-782 of human XPB (GeneID: 2071, also known as ERCC3), conjugated to KLH via an N-terminal added cysteine residue. The immunizing peptide differs from the mouse and rat sequences in one and three amino acids, respectively. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-XPB specifically recognizes XPB. Applications include immunoblotting (90 kDa) and immuno-fluorescence. Staining of the XPB band in immunoblotting is specifically inhibited by the immunizing peptide.

The integrity of genetic information depends on the fidelity of DNA replication and on the efficiency of several different DNA repair processes. The primary structure of DNA is constantly subjected to alteration by cellular metabolites and exogenous DNA-damaging agents, which cause alterations such as base changes of deletions, fusions, translocations or aneuploidy. The four types of response pathways elicited by DNA damage are DNA repair, DNA damage checkpoints, transcriptional response, and apoptosis. Defects in these pathways may cause genomic instability.^{1, 2} DNA repair mechanisms include direct repair, base excision repair, nucleotide excision repair, double-strand break repair, and cross-linking repair.¹⁻³ Nucleotide excision repair (NER) is the major repair system for removing bulky DNA lesions formed by exposure to UV light or environmental chemicals. The damaged bases are removed by a multisubunit enzyme system that makes dual incisions bracketing the lesion in the damaged strand.^{1, 4-6} The basic steps of NER are: (a) damage recognition, (b) dual incisions bracketing the lesion to form a 24-32-nt oligomer in eukaryotes, (c) release of the oligomer, (d) repair synthesis to fill in the resulting gap, and (e) ligation.¹ In human, excision repair is carried out by six repair factors, RPA, XPA, XPC, TFIIH, XPG, and XPF/ERCC1, composed of 15 polypeptides. Defects in excision repair cause a photosensitivity

syndrome called xeroderma pigmentosum (XP), which is characterized by a very high incidence of lightinduced skin cancer.^{7,8} Although the order of arrival of each factor at a lesion remains controversial, it is widely accepted that the XPC- hHR23B complex recognizes the DNA damage-induced helical distortion, and the transcription factor TFIIH, XPA (possibly in its homodimeric form), and replication protein A (RPA) arrived sequentially at the site of damage.^{9,10} TFIIH consists of nine protein subunits, XPB (ERCC3) being one of them. It is an ATP-dependent DNA helicase of 89 kDa. The C-terminal part of the protein is involved in DNA repair and transcription. Phosphorylation on Ser⁷⁵¹ of XPB inhibits TFIIH repair activity, leaving its transcription function intact.^{11, 12}

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Antibody concentration: ~1 mg/mL

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in "frostfree" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a working concentration of 1-2 $\mu g/mL$ is recommended using HepG2 cell lysates.

Indirect immunofluorescence: a working concentration of 10-20 μ g/mL is recommended using HeLa cells fixed with paraformaldehyde-Triton[®].

Recommendation: For immunoblotting, we strongly advise diluting the antibody in phosphate buffered saline (PBS) containing 1-3% non-fat dry milk and 0.05% TWEEN[®] 20.

Note: In order to obtain the best results using various techniques and preparations, we recommend determining the optimal working dilutions by titration.

References

- 1. Sancar, A., et al., *Annu. Rev. Biochem.*, **73**, 39-85 (2004).
- Ataian, Y., and Krebs, J.E., *Biochem. Cell Biol.*, 84, 490-504 (2006).
- 3. Lindahl, T., Nature, 362, 709-715 (1993).

- Wood, R.D., J. Biol. Chem., 272, 23465-23468 (1997).
- 5. Mu, D. et al., *J. Biol. Chem.*, **270**, 2425-2418 (1995).
- 6. Liu, Y., et al., Biochemistry, 44, 7361-7368 (2005).
- Sancar, A., and Reardon, J.T., *Adv. Protein Chem.*, 69, 43-71 (2004).
- 8. Neumann, A.S., et al., *Mol. Carcinog.*, **42**, 65-92 (2005).
- 9. Park, C.-J., and Choi, B-S. , *FEBS J.*, **273**, 1600-1608 (2006).
- 10. Mer, G., et al, Cell, et al., 103, 449-456 (2000).
- 11. Mounkes, L.C., et al., Cell, 71, 925-937 (1992).
- 12. Coin, F., et al., EMBO J., 23, 4385-4846 (2004).

Triton is registered trademark of the Dow Chemical Company TWEEN is registered trademark of Uniqema, a business unit of ICI Americas, Inc.

NV,KAA,PHC 02/07-1

Sigma brand products are sold through Sigma-Aldrich, Inc.