Anti-XPA antibody, monoclonal (5F12)

70-031 100 ug

Storage: Ship at 4℃ or -20℃, and store at -20℃. Do not freeze.

Immunogen : Recombinant full-length human XPA protein

Cross reactivity: human (expected to react also with mouse XPA from the sequence homology)

Applications

1. Western blotting (1/1,000~1/10,000)
2. Immunofluorescence staining (1/100~1/1,000)
3. ELISA
4. Inhibition of in vitro excision repair reaction
5. Inhibition of XPA interaction with ERCC1 and TFIIH

Other applications have not been tested.

Epitope: Amino acids 30-47

Subtype: IgG2b

Form: Purified IgG, 1 mg/ml in PBS pH 7.2, 50% glycerol, filter-sterilized

Background: XP (Xeroderma pigmentosum) is an autosomal recessive human disease characterized by hypersensitivity to sunlight and a high incidence of skin cancer on sun-exposed skin (1). Cells from XP patients are hypersensitive to killing by UV irradiation because of a defect in nucleotide excision repair (NER). XP is classified into seven complementation groups (A~G) and a variant form (1). XPA shows the most severe symptoms. Products encoded by the XP genes function in repairing UV-induced cyclobutane pyrimidine dimmer and (6-4) photoproducts as well as chemically induced variety of DNA lesions (1).

XPA protein consists of 273 amino acids and forms a complex with many proteins such as RPA, ERCC1, TFIIH、XAB1, and XAB2, which plays a role in early step of NER. The hybridoma 5F12 was constructed by Prof. K. Tanaka's group who first cloned the XPA gene (2, 3).

Data Link UniProtKB/Swiss-Prot P23025 (XPA_HUMAN)

References: This antibody is described and usedin Ref. 2

1. Friedberg EC *et al DNA Repair and Mutagenesis* 2nd ed., ASM Press (2006)
2. Saijo M *et al* "Inhibition of nucleotide excision repair by anti-XPA monoclonal antibodies which interefere with binding to RPA, ERCC1, and TFIIH" *Biochem Biophys Res Comm* **321**:815-822 (2004) PMID: 15358100
3. Tanaka K *et al* "Analysis of a human DNA excision repair gene involved in group A xeroderma pigmentosum and containing a zinc-finger domain" *Nature* **348**:73 -76 (1990) PMID: 2234061

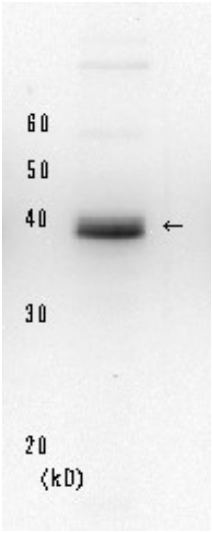

Fig. 1　Western blot of endogenous XpA protein.

Hela cell whole extrac (20　μg)t.

Antibody was used at 1/2,000 dilution.

As secondary antibody, HRP conjugated goat anti-mouse IgG was used at 1/20,000 dilution.

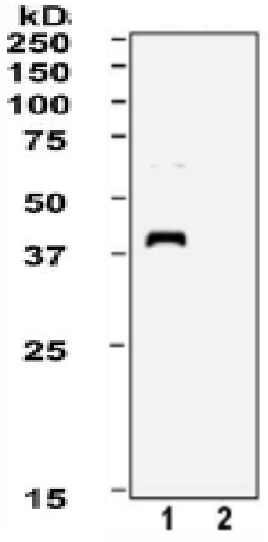

Fig 2　Weastern blot of XpA: Validation.

Lane 1. Extract of Hela cells (XpA wild type)

Lane 2. Extract of　XP12ROSV cells (XpA deficient)

The primary and the secondary antibodies were used at 1/2,000 and 1/20,000 dilutions.

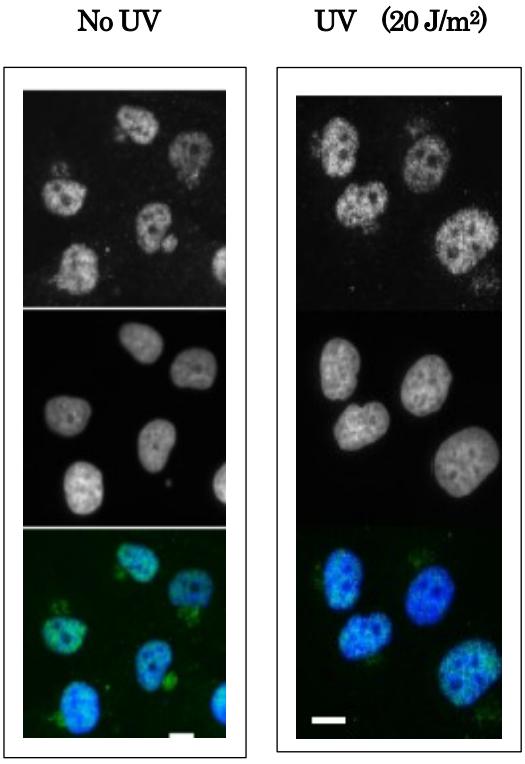

Fig.2　Immunofluorescence staining of human fibroblast cells (GM0637) using anti-XpA antibody (5F12)

The cells were non-irradiated (left) or irradiated with　UV at 20 J/m^2 and fixed after 30 min with paraform aldehyde. The antibody was used at 1/100 dilution and as the second antibody, Alexa 488 conjugated goat anti-mouse IgG was used at 1/5,000 dilution.