abcam

Product datasheet

Anti-FANCD2 antibody [EPR2302] ab108928



ייבעדיו RabMAb

★★★★★ 4 Abreviews 44 References 画像数 12

製品の概要

製品名 Anti-FANCD2 antibody [EPR2302]

製品の詳細 Rabbit monoclonal [EPR2302] to FANCD2

由来種 Rabbit

特異性 The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for

mouse and rat.

アプリケーション 適用あり: IHC-P, ICC/IF, IP, Flow Cyt (Intra), WB

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: HAP1, HeLa and HEK293 cell lysates. IHC-P: Human tonsil tissue. ICC/IF: HAP1 and HeLa

cells. Flow Cyt (intra): Jurkat cells. IP: HeLa cell lysate

特記事項 This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity - Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

精製度 Protein A purified

ポリモノ モノクローナル

lgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab108928の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

| アプリケーション | Abreviews | 特記事項 |
|------------------|-----------------|---|
| IHC-P | | 1/50 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols. The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat. |
| ICC/IF | **** <u>(1)</u> | 1/250 - 1/500. |
| IP | | 1/10 - 1/100. |
| Flow Cyt (Intra) | | 1/200. |
| WB | ★★★★★(3) | 1/1000 - 1/10000. Detects a band of approximately 155 kDa (predicted molecular weight: 166 kDa). |

ターゲット情報

組織特異性

機能 Required for maintenance of chromosomal stability. Promotes accurate and efficient pairing of

homologs during meiosis. Involved in the repair of DNA double-strand breaks, both by homologous recombination and single-strand annealing. May participate in S phase and G2 phase checkpoint activation upon DNA damage. Promotes BRCA2/FANCD1 loading onto

damaged chromatin. May also be involved in B-cell immunoglobulin isotype switching.

Highly expressed in germinal center cells of the spleen, tonsil, and reactive lymph nodes, and in the proliferating basal layer of squamous epithelium of tonsil, esophagus, oropharynx, larynx and cervix. Expressed in cytotrophoblastic cells of the placenta and exocrine cells of the pancreas (at

 $protein \, level). \, Highly \, expressed \, in \, test is, \, where \, expression \, is \, restricted \, to \, maturing$

spermatocytes.

関連疾患 Defects in FANCD2 are a cause of Fanconi anemia complementation group D type 2 (FANCD2)

[MIM:227646]. It is a disorder affecting all bone marrow elements and resulting in anemia, leukopenia and thrombopenia. It is associated with cardiac, renal and limb malformations, dermal pigmentary changes, and a predisposition to the development of malignancies. At the cellular level it is associated with hypersensitivity to DNA-damaging agents, chromosomal instability

(increased chromosome breakage) and defective DNA repair.

発生段階 Highly expressed in fetal oocytes, and in hematopoietic cells of the fetal liver and bone marrow (at

protein level).

ドメイン The C-terminal 24 residues of isoform 2 are required for its function.

翻訳後修飾 Monoubiquitinated on Lys-561 during S phase and upon genotoxic stress (isoform 1 and isoform

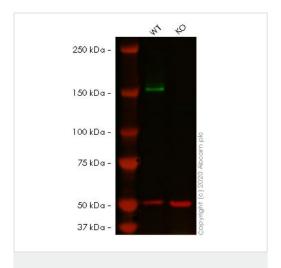
2). Deubiquitinated by USP1 as cells enter G2/M, or once DNA repair is completed.

Monoubiquitination requires the FANCA-FANCB-FANCC-FANCE-FANCF-FANCG-FANCM complex, RPA1 and ATR, and is mediated by FANCL/PHF9. Ubiquitination is required for binding to chromatin, interaction with BRCA1, BRCA2 and MTMR15/FAN1, DNA repair, and normal cell cycle progression, but not for phosphorylation on Ser-222 or interaction with MEN1. Phosphorylated in response to various genotoxic stresses by ATM and/or ATR. Upon ionizing radiation, phosphorylated by ATM on Ser-222 and Ser-1404. Phosphorylation on Ser-222 is required for S-phase checkpoint activation, but not for ubiquitination, foci formation, or DNA repair. In contrast, phosphorylation by ATR on other sites may be required for ubiquitination and foci formation.

細胞内局在

Nucleus. Concentrates in nuclear foci during S phase and upon genotoxic stress.

画像



Western blot - Anti-FANCD2 antibody [EPR2302] (ab108928)

All lanes : Anti-FANCD2 antibody [EPR2302] (ab108928) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: FANCD2 knockout HeLa cell lysate

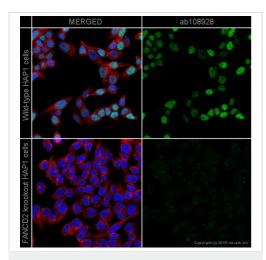
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 166 kDa Observed band size: 166 kDa

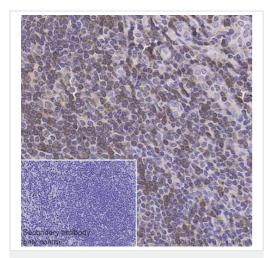
Lanes 1-2: Merged signal (red and green). Green - ab108928 observed at 166 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) observed at 50 kDa.

ab108928 was shown to react with FANCD2 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab261743 (knockout cell lysate ab257173) was used. Wild-type HeLa and FANCD2 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab108928 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



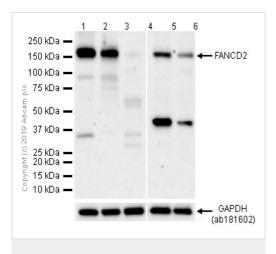
Immunocytochemistry/ Immunofluorescence - Anti-FANCD2 antibody [EPR2302] (ab108928)

ab108928 (unpurified) staining FANCD2 in wild-type HAP1 cells (top panel) and FANCD2 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10 minutes), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1 hour. The cells were then incubated with ab108928 at 1/250 dilution and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1 hour with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FANCD2 antibody
[EPR2302] (ab108928)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human tonsil tissue sections labeling FANCD2 with purified ab108928 at 1/50 dilution (3.6 µg/ml). Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-FANCD2 antibody [EPR2302] (ab108928)

All lanes: Anti-FANCD2 antibody [EPR2302] (ab108928) at 1/1000 dilution (Purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lane 2: MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates

Lane 3: C6 (Rat glial tumor glial cell) whole cell lysates

Lane 4 : RAW264.7 (Mouse Abelson murine leukemia virusinduced tumor macrophage) whole cell lysates

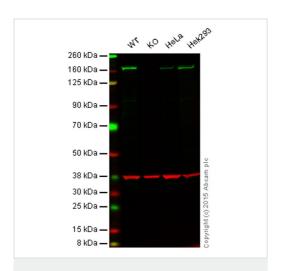
Lane 5: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 166 kDa **Observed band size:** 165 kDa



Western blot - Anti-FANCD2 antibody [EPR2302] (ab108928)

Lane 1: Wild-type HAP1 cell lysate (20 µg)

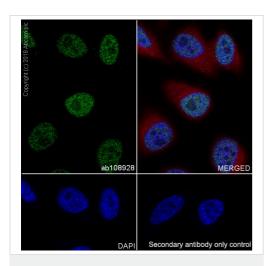
Lane 2: FANCD2 knockout HAP1 cell lysate (20 µg)

Lane 3: HeLa cell lysate (20 µg)

Lane 4: HEK293 cell lysate (20 µg)

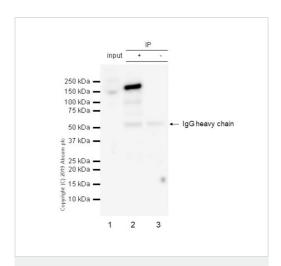
Lanes 1 - 4: Merged signal (red and green). Green - ab108928 (unpurified) observed at 165 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab108928 was shown to specifically react with FANCD2 when FANCD2 knockout samples were used. Wild-type and FANCD2 knockout samples were subjected to SDS-PAGE. ab108928 and <u>ab8245</u> (loading control to GAPDH) were diluted 1/1000 and 1/2000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-FANCD2 antibody [EPR2302] (ab108928)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling FANCD2 with purified ab108928 at 1/500 dilution (0.4 μ g/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) at 1/200 (2.5 μ g/ml) dilution. Goat anti rabbit lgG (Alexa Fluor[®] 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 μ g/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunoprecipitation - Anti-FANCD2 antibody [EPR2302] (ab108928)

ab108928 (purified) at 1/20 dilution (1ug) immunoprecipitating FANCD2 in HeLa whole cell lysates.

Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates 10ug

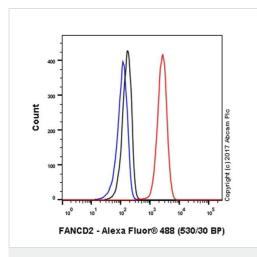
Lane 2 (+): ab108928 & HeLa whole cell lysates

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab108928 in HeLa whole cell lysates

For western blotting, VeriBlot for IP Detection Reagent (HRP)

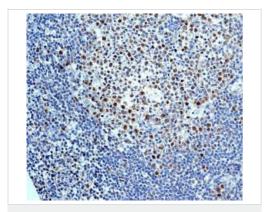
(<u>ab131366</u>) was used at 1/1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-FANCD2 antibody [EPR2302] (ab108928)

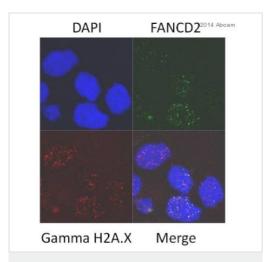
Intracellular Flow Cytometry analysis of Jurkat (human acute T cell leukemia) cells labeling FANCD2 (red) with ab108928 (purified) at a 1/200 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit lgG (Alexa Fluorr® 488) (ab150077) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal lgG (ab172730). Blue (unlabeled control) - Cells without incubation with primary and secondary antibodies.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-FANCD2 antibody
[EPR2302] (ab108928)

Immunohistochemical staining of paraffin-embedded human tonsil tissue using ab108928 (unpurified) at a dilution of 1/100.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

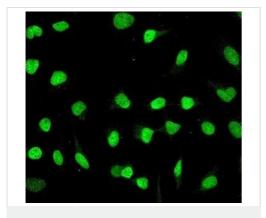


Immunocytochemistry/ Immunofluorescence - Anti-FANCD2 antibody [EPR2302] (ab108928)

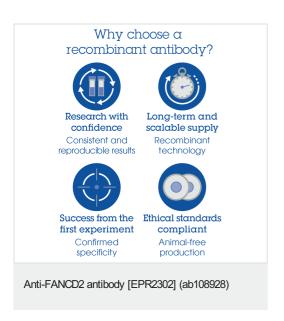
This image is courtesy of an anonymous Abreview

ab108928 (unpurified) staining FANCD2 in human U2OS osteosarcoma cells by ICC/IF

(Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.5% Triton X-100 and blocked with 10% goat serum for 1 hour at 20°C. Samples were incubated with primary antibody (1/300) for 18 hours at 4°C. An Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG monoclonal (1/1000) was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-FANCD2 antibody [EPR2302] (ab108928) HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained for FANCD2 (green) using ab108928 (unpurified) (1/250 dilution) in ICC/IF.



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