abcam

Product datasheet

Anti-BubR1 antibody [8G1] ab4637

★★★★☆ 6 Abreviews 28 References 画像数 4

製品の概要

製品名	Anti-BubR1 antibody [8G1]
製品の詳細	Mouse monoclonal [8G1] to BubR1
由来種	Mouse
アプリケーション	**適用あり:** IP, ICC/IF, WB
種交差性	**交差種:** Human
免疫原	N-terminal tagged fusion recombinant fragment, corresponding to amino acids 1-350 of Human BubR1.
ポジティブ・コントロール	Recent batches of this antibody have given a positive signal in WB against recombinant BubR1 however we have been unable to detect endogenous BubR1 in lysates tested. Previous batches of this antibody have given a positive signal in HeLa nuclear lysate (see tested notes section for protocol details). For further information please contact our Scientific Support Team

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 or -80°C. Avoid freeze / thaw cycle.
バッファー	Preservative: 0.1% Sodium azide Constituent: PBS
精製度	IgG fraction
特記事項（精製）	This antibody was produced as tissue culture supernatant and concentrated in Integra CL-1000 flasks. A 2 step PEG precipitation procedure was used to purify the antibody.
ポリ/モノ	モノクローナル
クローン名	8G1
アイソタイプ	IgG1

アプリケーション

The Abpromise guarantee	**Abpromise保証は、** 次のテスト済みアプリケーションにおけるab4637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
IP		Use at an assay dependent dilution.
ICC/IF	★★★★☆ **(6)**	1/200.
WB		Use a concentration of 10 µg/ml. Detects a band of approximately 130 kDa (predicted molecular weight: 120 kDa). Abcam recommends using milk as the blocking agent. We would suggest using the following protocol; arrest HeLa cells in mitosis with 60ng/ml of sterile nocodazole. Harvest cells by mechanical shake-off between 12 to 16 hours after drug addition. Pellet cells, wash in PBS, and lyse in RIPA. Centrifuge lysates at 4C/15,000g

ターゲット情報

機能

Essential component of the mitotic checkpoint. Required for normal mitosis progression. The mitotic checkpoint delays anaphase until all chromosomes are properly attached to the mitotic spindle. One of its checkpoint functions is to inhibit the activity of the anaphase-promoting complex/cyclosome (APC/C) by blocking the binding of CDC20 to APC/C, independently of its kinase activity. The other is to monitor kinetochore activities that depend on the kinetochore motor CENPE. Required for kinetochore localization of CENPE. Negatively regulates PLK1 activity in interphase cells and suppresses centrosome amplification. Also implicated in triggering apoptosis in polyploid cells that exit aberrantly from mitotic arrest. May play a role for tumor suppression.

組織特異性

Highly expressed in thymus followed by spleen. Preferentially expressed in tissues with a high mitotic index.

関連疾患

Note=Defects in BUB1B are associated with tumor formation.
Defects in BUB1B are the cause of premature chromatid separation trait (PCS) [MIM:176430]. PCS consists of separate and splayed chromatids with discernible centromeres and involves all or most chromosomes of a metaphase. It is found in up to 2% of metaphases in cultured lymphocytes from approximately 40% of normal individuals. When PCS is present in 5% or more of cells, it is known as the heterozygous PCS trait and has no obvious phenotypic effect, although some have reported decreased fertility. Inheritance is autosomal dominant.
Defects in BUB1B are the cause of mosaic variegated aneuploidy syndrome (MVA) [MIM:257300]. MVA is a severe autosomal recessive developmental disorder characterized by mosaic aneuploidies, predominantly trisomies and monosomies, involving multiple different chromosomes and tissues. The proportion of aneuploid cells varies but is usually more than 25% and is substantially greater than in normal individuals. Affected individuals typically present with severe intrauterine growth retardation and microcephaly. Eye anomalies, mild dysmorphism, variable developmental delay, and a broad spectrum of additional congenital abnormalities and medical conditions may also occur. The risk of malignancy is high, with rhabdomyosarcoma, Wilms tumor and leukemia reported in several cases. MVA is caused by biallelic mutations in the BUB1B gene.

配列類似性

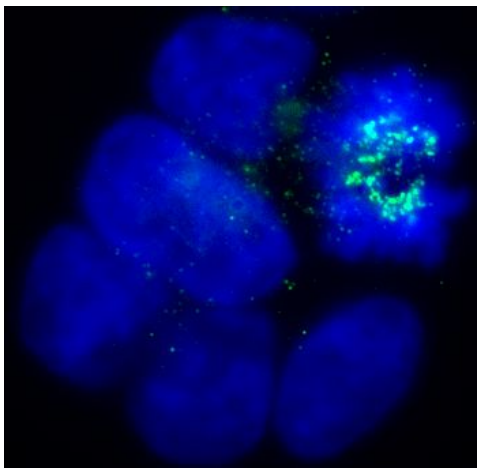
Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. BUB1 subfamily.
Contains 1 BUB1 N-terminal domain.
Contains 1 protein kinase domain.

ドメイン

The D-box targets the protein for rapid degradation by ubiquitin-dependent proteolysis during the transition from mitosis to interphase.

	The BUB1 N-terminal domain directs kinetochore localization and binding to BUB3.
翻訳後修飾	Proteolytically cleaved by caspase-3 in a cell cycle specific manner. The cleavage might be involved in the durability of the cell cycle delay. Caspase-3 cleavage is associated with abrogation of the mitotic checkpoint. The major site of cleavage is at Asp-610. Acetylation at Lys-250 regulates its degradation and timing in anaphase entry. Ubiquitinated. Degradated by the proteasome. Sumoylated by SUMO2 and SUMO3. The sumoylation mediates the association with CENPE at the kinetochore. Autophosphorylated in vitro. Intramolecular autophosphorylation is stimulated by CENPE. Phosphorylated during mitosis and hyperphosphorylated in mitotically arrested cells. Phosphorylation at Ser-670 and Ser-1043 occurs at kinetochores upon mitotic entry with dephosphorylation at the onset of anaphase.
細胞内局在	Cytoplasm. Nucleus. Chromosome > centromere > kinetochore. Cytoplasm > cytoskeleton > centrosome. Cytoplasmic in interphase cells. Associates with the kinetochores in early prophase. Kinetochore localization requires BUB1, PLK1 and CASC5.

画像

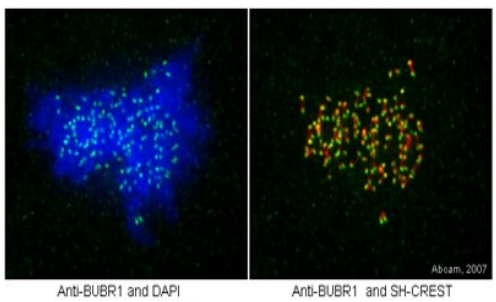


Immunocytochemistry/ Immunofluorescence - Anti-BubR1 antibody [8G1] (ab4637)

Luke Hughes-Davies and Rhiannon Jade, Gurdon Institute, Cambridge, UK

Immunofluorescent imaging of an asynchronous cycling population of human cells (U2OS) with ab4637 strikingly confirms the specificity of this antibody. No signal is detected from interphase cells, whereas cells undergoing mitosis accumulate BubR1 at the kinetochores. Image reveals kinetochores at prometaphase, and exactly agrees with numerous published images of BubR1 (see for example Johnson et al J Cell Sci. 117(Pt 8):1577-89 (2004).

IF was performed with a standard paraformaldehyde technique (fixed in PBS buffered PFH 4% for 5 minutes, permeabilised with 0.5% triton-PBS for 5 minutes, blocked with 5% milk / 0.2% tween for one hour. Primary antibody used at 1/100 in 5% milk / 0.2% TWEEN for one hour, secondary antibody for 30 minutes. All blocking and incubation steps carried out at 37 degrees C.

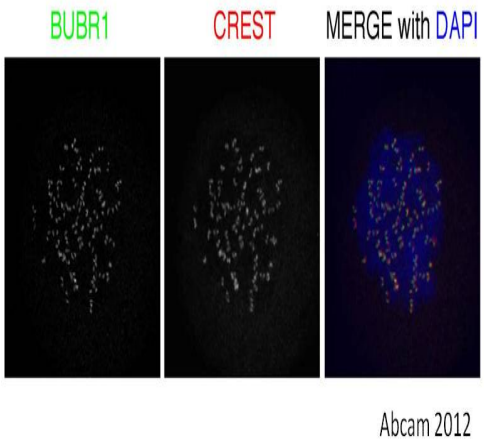


Immunocytochemistry/ Immunofluorescence - Anti-BubR1 antibody [8G1] (ab4637)

This image is courtesy of Scott Slattery and Mike Mancini

HeLa cells were stained with anti-BubR1 (ab4637) in green and DAPI in blue in panel one and anti-BubR1 (green) and SH-CREST (red) to stain the centromeres in panel 2. Fix cells for 30 minutes on ice in 4% formaldehyde in PEM. Quench autofluorescence 2 x 5 min. with 1 mg/ml Na borohydride OR 100 mM ammonium chloride in PEM. Permeablize 30 min. with 0.5% TX-100 in PEM. Block 30 minutes in 5% milk in TBST. Primary antibody was incubated 1/200 overnight at 4oC diluted in 5% milk in TBST. Secondary antibody was incubated for 1 hour at RT diluted in 5% milk in TBST. Post-fix 20 min. on ice in 4% formaldehyde in PEM. Quench autofluorescence 2 x 5 min. with ammonium chloride in PEM.

Counterstain with DAPI in TBST. Mount with ProLong Gold antifade reagent from Invitrogen. Notes: Ample washing between each step. TBST = Tris buffered saline + 0.1% Tween. PEM = 80 mM K-PIPES, pH 6.8, 5 mM EGTA, 2 mM MgCl2.



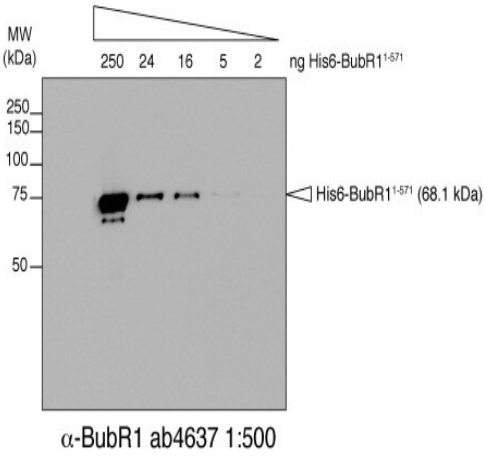
Immunocytochemistry/ Immunofluorescence - Anti-BubR1 antibody [8G1] (ab4637)

This image is courtesy of an anonymous abreview.

Immunofluorescence of nocodazole treated HeLa cells with ab4637 staining BubR1 (green). CREST was used to mark centromeres (red) and the DNA is stained with DAPI (blue).

Cells were fixed with PFA 4% and permealized with Triton X-100 0.1%. Blocking was done with 4% BSA. ab4637 was used at a 1:200 dilution in 4% BSA for 1.5h at room temperature. Secondary antibodies were incubated for 45min. Coverslips were then stained with DAPI and mounted with Mowiol mounting media.

ab4637 reveals BubR1 in mitotic cells colocalizing with kinetochores, as expected.



Western blot - Anti-BubR1 antibody [8G1] (ab4637)

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