abcam

Product datasheet

Anti-Eg5 (phospho T927) antibody ab61104

★★★★★ 1 Abreviews 3 Images

Overview

Product name	Anti-Eg5 (phospho T927) antibody
Description	Rabbit polyclonal to Eg5 (phospho T927)
Host species	Rabbit
Specificity	ab61104 detects endogenous levels of Eg5 only when phosphorylated at threonine 927.
Tested applications	**Suitable for:** WB, ICC/IF, ELISA, IHC-P
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse
Immunogen	Synthetic phosphopeptide derived from human Eg5 around the phosphorylation site of threonine 927 (G-T-T^{P}-P-Q).
Positive control	Human lung carcinoma tissue, extracts from COLO205 cells.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	Preservative: 0.02% Sodium Azide Constituents: 50% Glycerol, PBS, 150mM Sodium chloride, pH 7.4
Purity	Immunogen affinity purified
Purification notes	Affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatography using non-phosphopeptide corresponding to the phosphorylation site.
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab61104** in the following tested applications.

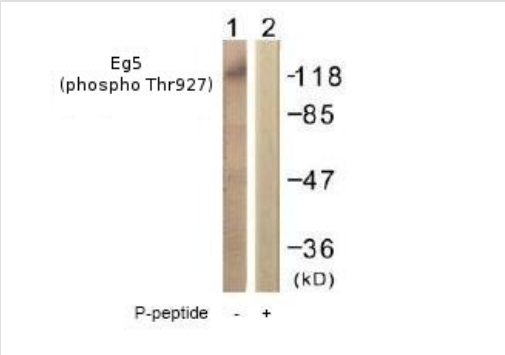
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★	1/500 - 1/1000. Detects a band of approximately 120 kDa (predicted molecular weight: 120 kDa).
ICC/IF		1/500 - 1/1000.
ELISA		1/5000.
IHC-P		Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Target

Function	Motor protein required for establishing a bipolar spindle. Blocking of KIF11 prevents centrosome migration and arrest cells in mitosis with monoastral microtubule arrays.
Involvement in disease	Defects in KIF11 are the cause of microcephaly with or without chorioretinopathy, lymphedema, or mental retardation (MCLMR) [MIM:152950]. An autosomal dominant disorder that involves an overlapping but variable spectrum of central nervous system and ocular developmental anomalies. Microcephaly ranges from mild to severe and is often associated with mild to moderate developmental delay and a characteristic facial phenotype with upslanting palpebral fissures, broad nose with rounded tip, long philtrum with thin upper lip, prominent chin, and prominent ears. Chorioretinopathy is the most common eye abnormality, but retinal folds, microphthalmia, and myopic and hypermetropic astigmatism have also been reported, and some individuals have no overt ocular phenotype. Congenital lymphedema, when present, is typically confined to the dorsa of the feet, and lymphoscintigraphy reveals the absence of radioactive isotope uptake from the webspaces between the toes.
Sequence similarities	Belongs to the kinesin-like protein family. BimC subfamily. Contains 1 kinesin-motor domain.
Post-translational modifications	Phosphorylated exclusively on serine during S phase, but on both serine and Thr-926 during mitosis, so controlling the association of KIF11 with the spindle apparatus (probably during early prophase). Phosphorylated upon DNA damage, probably by ATM or ATR. A subset of this protein primarily localized at the spindle pole is phosphorylated by NEK6 during mitosis; phosphorylation is required for mitotic function.
Cellular localization	Cytoplasm. Cytoplasm > cytoskeleton > spindle pole.

Images

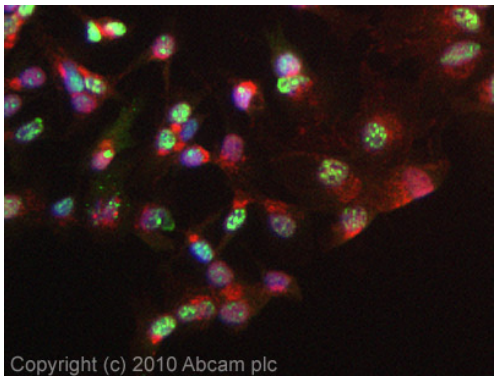


Western blot - Eg5 (phospho T927) antibody (ab61104)

All lanes : Anti-Eg5 (phospho T927) antibody (ab61104) at 1/500 dilution

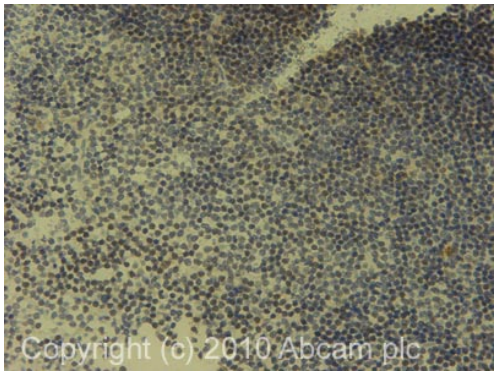
Lane 1 : Extracts from COLO205 cells.
Lane 2 : Extracts from COLO205 cells with the immunising peptide

Predicted band size: 120 kDa
Observed band size: 120 kDa



Immunocytochemistry/ Immunofluorescence-Eg5 (phospho T927) antibody(ab61104)

ICC/IF image of ab61104 stained HepG2 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab61104, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections)-Eg5 (phospho T927) antibody(ab61104)

IHC image of ab61104 staining in human bone marrow chronic lymphocytic leukemia formalin fixed paraffin embedded tissue section, performed on a Leica Bond^{TM} system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab61104, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

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