# abcam

### Product datasheet

## Anti-TPX2 antibody [EPR23180-4] ab252945

Recombinant RabMAb

#### 1 References 10 Images

#### Overview

Product name	Anti-TPX2 antibody [EPR23180-4]	
Description	Rabbit monoclonal [EPR23180-4] to TPX2	
Host species	Rabbit	
Tested applications	Suitable for: Flow Cyt (Intra), ICC/IF, WB, IHC-P, IP	
Species reactivity	Reacts with: Human	
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.	
Positive control	WB: Raji, HEK-293 and HeLa whole cell lysate. IHC-P: Human lung cancer, breast cancer and colon cancer tissue. ICC/IF: HeLa and HEK-293 cells. Flow Cyt (intra): HeLa and Raji cells. IP: Raji whole cell lysate.	
General notes	<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> </ul>	

Properties	
Form	Liquid
Storage instructions	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.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR23180-4

#### Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab252945 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
Flow Cyt (Intra)		1/500.
ICC/IF		1/100.
WB		1/1000. Detects a band of approximately 100 kDa (predicted molecular weight: 86 kDa).
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/30.

#### Target

Function	Spindle assembly factor. Required for normal assembly of mitotic spindles. Required for normal assembly of microtubules during apoptosis. Required for chromatin and/or kinetochore dependent microtubule nucleation. Mediates AURKA localization to spindle microtubules. Activates AURKA by promoting its autophosphorylation at 'Thr-288' and protects this residue against dephosphorylation.
Tissue specificity	Expressed in lung carcinoma cell lines but not in normal lung tissues.
Sequence similarities	Belongs to the TPX2 family.
Developmental stage	Exclusively expressed in proliferating cells from the transition G1/S until the end of cytokinesis.
Post-translational modifications	Phosphorylated upon DNA damage, probably by ATM or ATR.
Cellular localization	Nucleus. Cytoplasm > cytoskeleton > spindle. Cytoplasm > cytoskeleton > spindle pole. During mitosis it is strictly associated with the spindle pole and with the mitotic spindle, whereas during S and G2, it is diffusely distributed throughout the nucleus. Is released from the nucleus in apoptotic cells and is detected on apoptotic microtubules.



Western blot - Anti-TPX2 antibody [EPR23180-4] (ab252945)

All lanes : Anti-TPX2 antibody [EPR23180-4] (ab252945) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate

Lane 2 : HEK-293 (human embryonic kidney epithelial cell), whole cell lysate

Lane 3 : Raji (human Burkitt's lymphoma B lymphocyte), whole cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

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

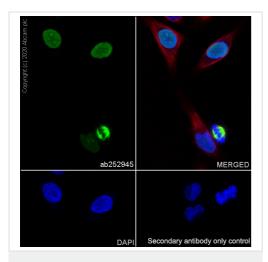
Predicted band size: 86 kDa Observed band size: 100 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The molecular weight observed is consistent with what has been described in the literature (PMID:25239289, 16489064).

Lysates should be made freshly and used in WB immediately to minimize protein degradation.

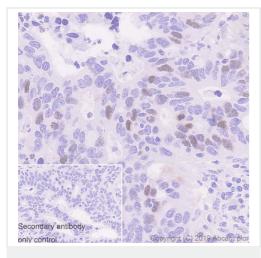
Exposure time: 5 secs.



Immunocytochemistry/ Immunofluorescence - Anti-TPX2 antibody [EPR23180-4] (ab252945)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa cells labeling TPX2 with ab252945 at 1/100 dilution, followed by <u>ab150077</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) antibody at 1/1000 dilution (Green). Confocal image showing positive staining in HeLa cells. <u>ab195889</u> Anti-alpha Tubulin antibody (Alexa Fluor<sup>®</sup> 594) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) at 1/1000 dilution.

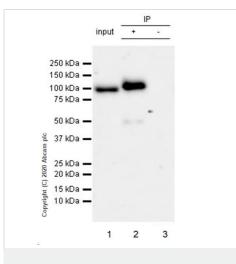


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TPX2 antibody [EPR23180-4] (ab252945)

Immunohistochemical analysis of paraffin-embedded human colon cancer tissue labeling TPX2 with ab252945 at 1/4000 followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining in proliferative cells of human colon cancer is observed(PMID:23634259, 22207630). The section was incubated with ab252945 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument Counterstained with hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.





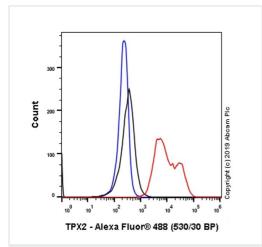
TPX2 was immunoprecipitated from 0.35 mg Raji (human Burkitt's lymphoma B lymphocyte) whole cell lysate with ab252945 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab252945 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: Raji whole cell lysate 10 µg.

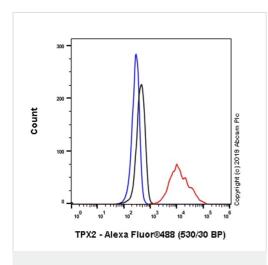
Lane 2: ab252945 IP in Raji whole cell lysate.

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab252945 in Raji whole cell lysate.

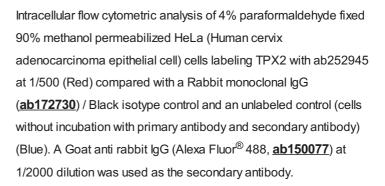
Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 41 secs.

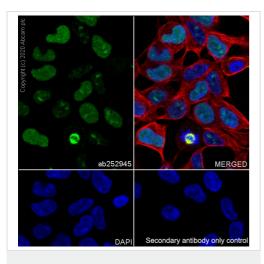


Flow Cytometry (Intracellular) - Anti-TPX2 antibody [EPR23180-4] (ab252945) Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Raji (Human Burkitt's lymphoma B lymphocyte) cells labeling TPX2 with ab252945 at 1/500 (Red) compared with a Rabbit monoclonal IgG (<u>ab172730</u>) / Black isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor<sup>®</sup> 488, <u>ab150077</u>) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-TPX2 antibody [EPR23180-4] (ab252945)

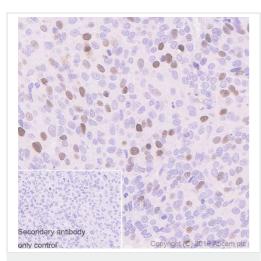




Immunocytochemistry/ Immunofluorescence - Anti-TPX2 antibody [EPR23180-4] (ab252945)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293 cells labeling TPX2 with ab252945 at 1/100 dilution, followed by <u>ab150077</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) antibody at 1/1000 dilution (Green). Confocal image showing positive staining in HEK-293 cells. <u>ab195889</u> Anti-alpha Tubulin antibody (Alexa Fluor<sup>®</sup> 594) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) at 1/1000 dilution.

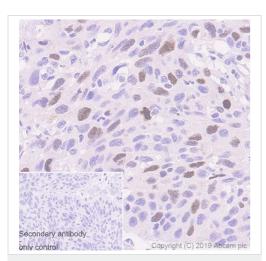


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TPX2 antibody [EPR23180-4] (ab252945)

Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling TPX2 with ab252945 at 1/4000 followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining in proliferative cells of human breast cancer is observed (PMID:31494045). The section was incubated with ab252945 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument Counterstained with hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

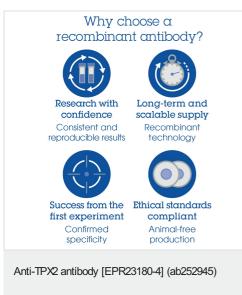


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TPX2 antibody [EPR23180-4] (ab252945)

Immunohistochemical analysis of paraffin-embedded human lung cancer tissue labeling TPX2 with ab252945 at 1/4000 followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining in proliferative cells of human lung cancer tissue is observed (PMID:23634259, 16489064). The section was incubated with ab252945 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument Counterstained with hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



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