abcam

Product datasheet

Anti-PABP antibody [10E10] ab6125

2 Abreviews 24 References 4 Images

Overview

Product name	Anti-PABP antibody [10E10]
Description	Mouse monoclonal [10E10] to PABP
Host species	Mouse
Tested applications	**Suitable for:** ELISA, WB, IP, ICC/IF, Flow Cyt
Species reactivity	**Reacts with:** Rabbit, Chicken, Human, Xenopus laevis **Does not react with:** Mouse, Drosophila melanogaster
Immunogen	Recombinant PABP (Human) expressed from its 1.85 kbp cDNA, NcoI to SspI.

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.1% Sodium azide Constituent: PBS
Purity	Protein A purified
Purification notes	Purified from supernatant.
Clonality	Monoclonal
Clone number	10E10
Myeloma	Sp2/0
Isotype	IgG2b

Applications

Our Abpromise guarantee covers the use of **ab6125** 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
ELISA		Use at an assay dependent concentration.

Application	Abreviews	Notes
WB	★★★★★	Use at an assay dependent concentration. Detects a band of approximately 70 kDa (predicted molecular weight: 71 kDa).
IP		Use at an assay dependent concentration.
ICC/IF	★★☆☆☆	Use at an assay dependent concentration. PubMed: 17977970
Flow Cyt		Use 1µg for 10^6 cells. ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.

Target

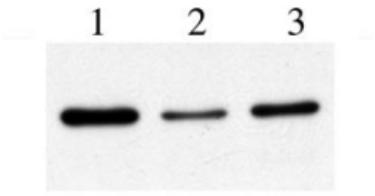
Relevance

The poly(A)-binding protein (PABP), which is found complexed to the 3-prime poly(A) tail of eukaryotic mRNA, is required for poly(A) shortening and translation initiation. Grange et al. (1987) isolated a melanoma cell cDNA encoding human PABP. The predicted 633-amino acid protein contains 4 repeats of an approximately 80-amino acid unit in its N-terminal half. The authors found that this repeat region is highly conserved between human and yeast PABP and is sufficient for poly(A) binding. In vitro translation of the human PABP cDNA yielded a protein with an apparent molecular mass of 73 kD by SDS-PAGE. Northern blot analysis indicated that PABP is expressed as a 2.9-kb mRNA in human melanoma cells. Gorlach et al. (1994) noted that each of the 4 repeats of PABP is a ribonucleoprotein (RNP) consensus sequence RNA-binding domain. They determined that PABP has a pI of approximately 10.3 and is a very abundant, stable protein. Immunofluorescence studies of mammalian cells indicated that PABP is located exclusively in the cytoplasm. However, using both indirect immunofluorescence and tagging of PABP1 by fusion to the green fluorescent protein (GFP), Afonina et al. (1998) demonstrated that PABP1 shuttles between the nucleus and cytoplasm. PABP1 accumulated in the nucleus when transcription was inhibited, suggesting that active transcription is required for nuclear export of PABP1.

Cellular localization

Cytoplasmic. Shuttles between the cytoplasm and the nucleus.

Images



Western blot - Anti-PABP antibody [10E10] (ab6125)

This image is courtesy of an abreview submitted by Francisco Ramirez-Valle

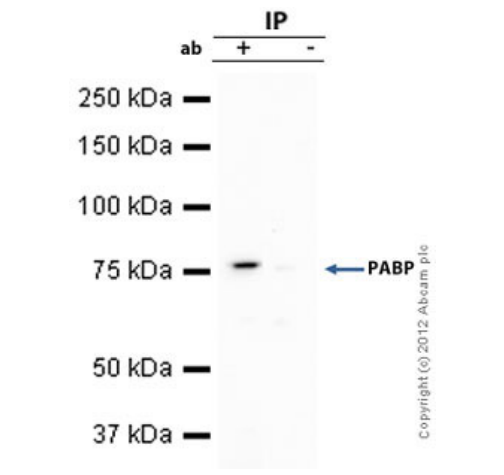
All lanes : Anti-PABP antibody [10E10] (ab6125) at 1/2000 dilution

Lane 1 : 10ug of protein from MCF10A cells transfected with negative control siRNA.
Lane 2 : 10ug of protein from MCF10A cells transfected with PABP specific siRNA.
Lane 3 : 10 ug of protein from MCF10A cells transfected with PABP specific siRNA.

Secondary
All lanes : Goat anti-mouse 1/10000

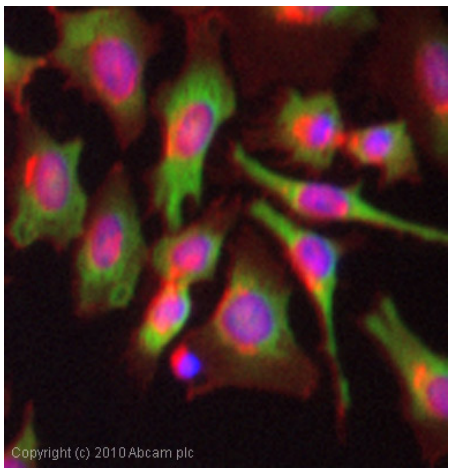
Predicted band size: 71 kDa
Observed band size: 70 kDa

The cells were lysed with NP40 buffer with protease inhibitor cocktail, 10 micrograms of protein were separated by SDS-PAGE and transferred to PVDF membrane, blocked and blotted for two hours with PABP antibody in TBST, washed three times, secondary antibody for 1 hr (goat anti-mouse, Amersham, 1:10000).



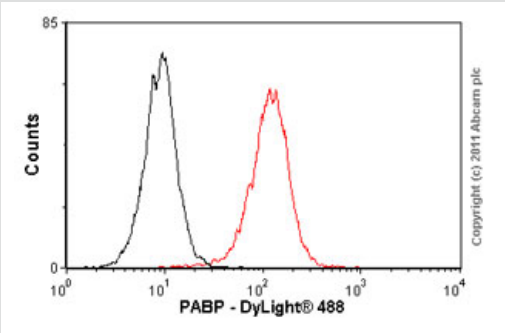
Immunoprecipitation - Anti-PABP antibody [10E10] (ab6125)

PABP was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Mouse monoclonal to PABP (ab6125) and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{O}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab6125.
Secondary: Goat polyclonal to mouse IgG light chain specific (HRP) at 1/5000 dilution.
Band: 76kDa: PABP; 25kDa.



Immunocytochemistry/ Immunofluorescence - Anti-PABP antibody [10E10] (ab6125)

ICC/IF image of ab6125 stained HeLa 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 (ab6125, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse 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.



Flow Cytometry - Anti-PABP antibody [10E10] (ab6125)

Overlay histogram showing HeLa cells stained with ab6125 (red line). The cells were fixed with 100% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab6125, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.

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