10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

Anti-CPSF6 Antibody Picoband™ (monoclonal, 3F11E1)

Catalog # ABO16592

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	Q16630
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Rat, Human, Mouse, Monkey
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-CPSF6 Antibody Picoband™ (monoclonal, 3F11E1) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat, Monkey.
Reconstitution	Adding 0.2 ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	11052
Other Names	Cleavage and polyadenylation specificity factor subunit 6, Cleavage and polyadenylation specificity factor 68 kDa subunit, CPSF 68 kDa subunit, Cleavage factor Im complex 68 kDa subunit, CFIm68, Pre-mRNA cleavage factor Im 68 kDa subunit, Protein HPBRII-4/7, CPSF6 (HGNC:13871)
Calculated MW	59210
Application Details	Western blot, 0.25-0.5 □g/ml, Human, Mouse, Rat, Monkey Immunohistochemistry(Paraffin-embedded Section), 2-5 □g/ml, Human, Mouse, Rat Immunocytochemistry/Immunofluorescence, 5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
Clone Names	Clone: 3F11E1
Immunogen	E.coli-derived human CPSF6 recombinant protein (Position: R50-Q176).
Purification	Immunogen affinity purified.
Storage	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.

Protein Information

Name	CPSF6 (HGNC:13871)
Function	Component of the cleavage factor Im (CFIm) complex that functions as an activator of the pre-mRNA 3'-end cleavage and polyadenylation processing required for the maturation of pre-mRNA into functional mRNAs (PubMed:14690600, PubMed:29276085, PubMed:8626397, PubMed:9659921). CFIm contributes to the recruitment of multiprotein complexes on specific sequences on the pre-mRNA 3'-end, so called cleavage and polyadenylation signals (pA signals) (PubMed:14690600, PubMed:8626397, PubMed:9659921). Most pre-mRNAs contain multiple pA signals, resulting in alternative cleavage and polyadenylation (APA) producing mRNAs with variable 3'-end formation (PubMed:23187700, PubMed:29276085). The CFIm complex acts as a key regulator of cleavage and polyadenylation site choice during APA through its binding to 5'- UGUA-3' elements localized in the 3'-untranslated region (UTR) for a huge number of pre-mRNAs (PubMed:20695905, PubMed:29276085). CPSF6 enhances NUDT21/CPSF5 binding to 5'-UGUA-3' elements localized upstream of pA signals and promotes RNA looping, and hence activates directly the mRNA 3'-processing machinery (PubMed:15169763, PubMed:21295486, PubMed:29276085). Plays a role in mRNA export (PubMed:19864460).
Cellular Location	Nucleus. Nucleus, nucleoplasm. Nucleus speckle. Cytoplasm. Note=Shuttles between the nucleus and the cytoplasm in a transcription- and XPO1/CRM1-independent manner, most probably in complex with the cleavage factor Im complex (CFIm) (PubMed:19864460). Colocalizes with PSPC1 in punctate subnuclear structures often located adjacent to nuclear speckles, called paraspeckles, and corresponding to interchromatin granules-associated zones (IGAZs) (PubMed:17267687). Distribution in speckles and paraspeckles varies during the cell cycle (PubMed:17267687). Associates at sites of active transcription on nascent perichromatin fibrils (PFs) and perichromatin granules (PubMed:17267687). Nuclear import is mediated via interaction with TNPO3 independently of CPSF6 phosphorylation status (PubMed:30916345).

Background

Cleavage and polyadenylation specificity factor subunit 6 is a protein that in humans is encoded by the CPSF6 gene. The protein encoded by this gene is one subunit of a cleavage factor required for 3' RNA cleavage and polyadenylation processing. The interaction of the protein with the RNA is one of the earliest steps in the assembly of the 3' end processing complex and facilitates the recruitment of other processing factors. The cleavage factor complex is composed of four polypeptides. This gene encodes the 68kD subunit. It has a domain organization reminiscent of spliceosomal proteins.

Images

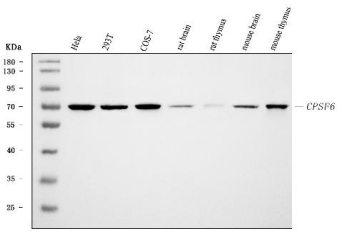


Figure 1. Western blot analysis of CPSF6 using anti-CPSF6 antibody (M04551).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours.
The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates,
Lane 2: human 293T whole cell lysates,

Lane 3: monkey COS-7 whole cell lysates,
Lane 4: rat brain tissue lysates,
Lane 5: rat thymus tissue lysates,
Lane 6: mouse brain tissue lysates,
Lane 7: mouse thymus tissue lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-CPSF6 antigen affinity purified monoclonal antibody (Catalog # M04551) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for CPSF6 at approximately 68 kDa. The expected band size for CPSF6 is at 59 kDa.

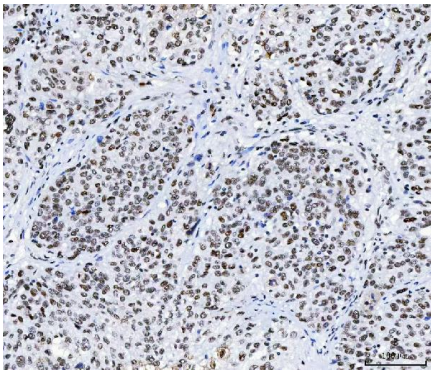


Figure 2. IHC analysis of CPSF6 using anti-CPSF6 antibody (M04551).
CPSF6 was detected in a paraffin-embedded section of human squamous cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-CPSF6 Antibody (M04551) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

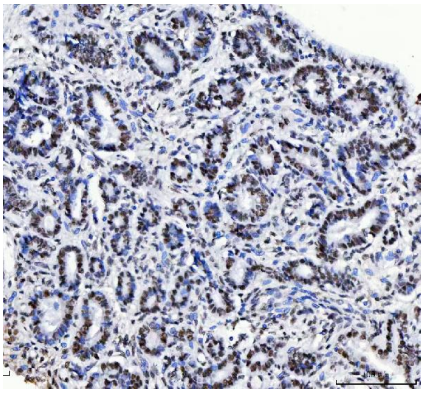


Figure 3. IHC analysis of CPSF6 using anti-CPSF6 antibody (M04551).
CPSF6 was detected in a paraffin-embedded section of human cervical cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-CPSF6 Antibody (M04551) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

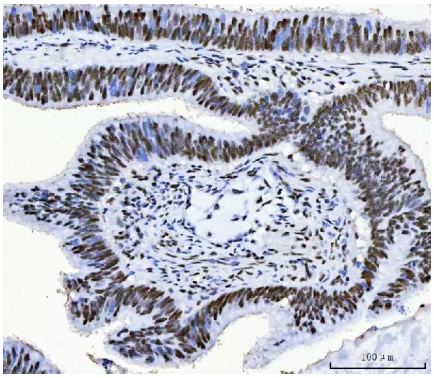


Figure 4. IHC analysis of CPSF6 using anti-CPSF6 antibody (M04551).
CPSF6 was detected in a paraffin-embedded section of human colorectal adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-CPSF6 Antibody (M04551) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was

developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

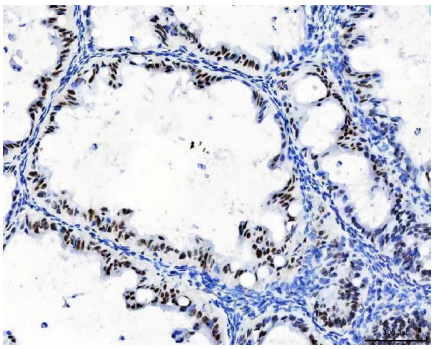


Figure 5. IHC analysis of CPSF6 using anti-CPSF6 antibody (M04551).
CPSF6 was detected in a paraffin-embedded section of human ovarian cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-CPSF6 Antibody (M04551) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.



Figure 6. IHC analysis of CPSF6 using anti-CPSF6 antibody (M04551).
CPSF6 was detected in a paraffin-embedded section of human spleen tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-CPSF6 Antibody (M04551) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

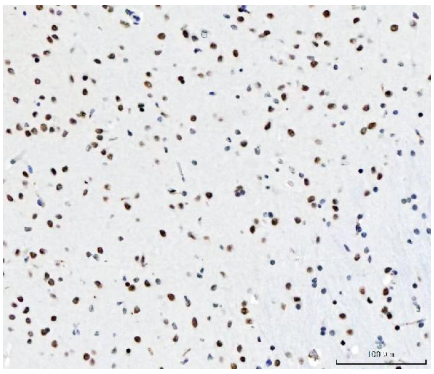


Figure 7. IHC analysis of CPSF6 using anti-CPSF6 antibody (M04551).
CPSF6 was detected in a paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-CPSF6 Antibody (M04551) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

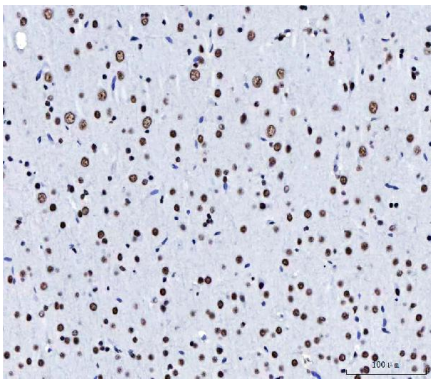


Figure 8. IHC analysis of CPSF6 using anti-CPSF6 antibody (M04551).
CPSF6 was detected in a paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-CPSF6 Antibody (M04551) overnight at 4°C. Peroxidase Conjugated Goat Anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Mouse IgG Super Vision Assay Kit (Catalog # SV0001) with DAB as the chromogen.

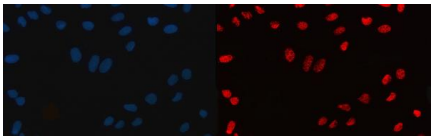


Figure 9. IF analysis of CPSF6 using anti-CPSF6 antibody (M04551).
CPSF6 was detected in an immunocytochemical section of U20S cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-CPSF6 Antibody (M04551) overnight at 4°C. Cy3 Conjugated Goat Anti-Mouse IgG (BA1031) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

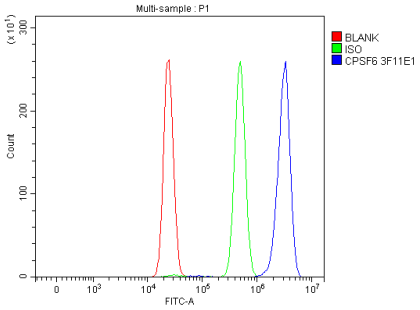


Figure 10. Flow Cytometry analysis of HepG2 cells using anti-CPSF6 antibody (M04551).
Overlay histogram showing HepG2 cells stained with M04551 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-CPSF6 Antibody (M04551, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.