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Anti-U2AF65/U2AF2 Picoband™ Antibody (monoclonal, 3G9)

Catalog # ABO15009

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P26368
Host	Mouse
Isotype	Mouse IgG2a
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-U2AF65/U2AF2 Picoband™ Antibody (monoclonal, 3G9) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	11338
Other Names	Splicing factor U2AF 65 kDa subunit, U2 auxiliary factor 65 kDa subunit, hU2AF(65), hU2AF65, U2 snRNP auxiliary factor large subunit, U2AF2, U2AF65
Calculated MW	53501
Application Details	Western blot, 0.1-0.25 □g/ml, Human, Mouse, Rat 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 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 3G9
Immunogen	E.coli-derived human U2AF65/U2AF2 recombinant protein (Position: M238-H470).
Purification	Immunogen affinity purified.
Storage	Store 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 freeze-thaw cycles.

Protein Information

Name	U2AF2
Synonyms	U2AF65
Function	Plays a role in pre-mRNA splicing and 3'-end processing (PubMed: 17024186). By recruiting PRPF19 and the PRP19C/Prp19 complex/NTC/Nineteen complex to the RNA polymerase II C-terminal domain (CTD), and thereby pre-mRNA, may couple transcription to splicing (PubMed:21536736). Induces cardiac troponin-T (TNNT2) pre-mRNA exon inclusion in muscle. Regulates the TNNT2 exon 5 inclusion through competition with MBNL1. Binds preferentially to a single-stranded structure within the polypyrimidine tract of TNNT2 intron 4 during spliceosome assembly. Required for the export of mRNA out of the nucleus, even if the mRNA is encoded by an intron-less gene. Represses the splicing of MAPT/Tau exon 10. Positively regulates pre-mRNA 3'-end processing by recruiting the CFIm complex to cleavage and polyadenylation signals (PubMed:17024186).
Cellular Location	Nucleus.

Background

Splicing factor U2AF 65 kDa subunit is a protein that in humans is encoded by the U2AF2 gene. It is mapped to 19q13.42. U2 auxiliary factor (U2AF), comprised of a large and a small subunit, is a non-snRNP protein required for the binding of U2 snRNP to the pre-mRNA branch site. This gene encodes the U2AF large subunit which contains a sequence-specific RNA-binding region with 3 RNA recognition motifs and an Arg/Ser-rich domain necessary for splicing. The large subunit binds to the polypyrimidine tract of introns early during spliceosome assembly. Multiple transcript variants have been detected for this gene, but the full-length natures of only two have been determined to date.

Images

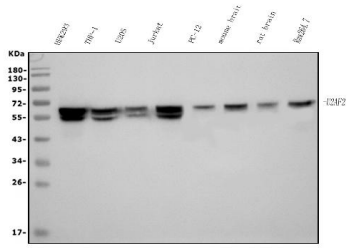


Figure 1. Western blot analysis of U2AF65/U2AF2 using anti-U2AF65/U2AF2 antibody (M03639-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human HEK293 whole cell lysates,
Lane 2: human THP-1 whole cell lysates,
Lane 3: human U20S whole cell lysates,
Lane 4: human Jurkat whole cell lysates,
Lane 5: rat PC-12 whole cell lysates,
Lane 6: mouse brain tissue lysates,
Lane 7: rat brain tissue lysates,
Lane 8: mouse RAW264.7 whole cell lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA 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-U2AF65/U2AF2 antigen affinity purified monoclonal antibody (Catalog # M03639-1) at 0.25 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 U2AF65/U2AF2 at approximately 65KD. The expected band size for U2AF65/U2AF2 is at 65KD.

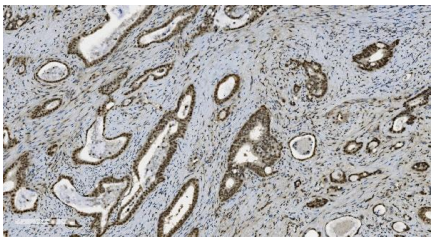


Figure 2. IHC analysis of U2AF65/U2AF2 using anti U2AF65/U2AF2 antibody (M03639-1).
U2AF65/U2AF2 was detected in paraffin-embedded section of human gallbladder adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-U2AF65/U2AF2 Antibody (M03639-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

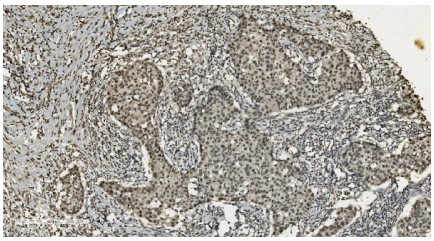


Figure 3. IHC analysis of U2AF65/U2AF2 using anti U2AF65/U2AF2 antibody (M03639-1).
U2AF65/U2AF2 was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-U2AF65/U2AF2 Antibody (M03639-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

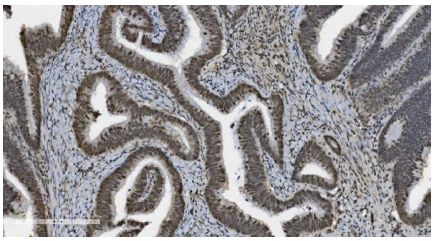


Figure 4. IHC analysis of U2AF65/U2AF2 using anti U2AF65/U2AF2 antibody (M03639-1).
U2AF65/U2AF2 was detected in paraffin-embedded section of human colonic adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-U2AF65/U2AF2 Antibody (M03639-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

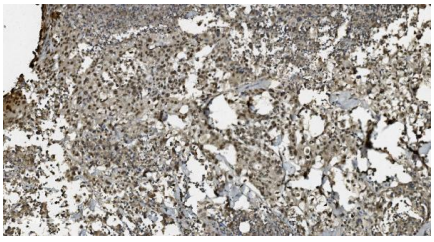


Figure 5. IHC analysis of U2AF65/U2AF2 using anti U2AF65/U2AF2 antibody (M03639-1).
U2AF65/U2AF2 was detected in paraffin-embedded section of human pancreatic cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-U2AF65/U2AF2 Antibody (M03639-1) overnight at 4°C. Biotinylated goat

anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

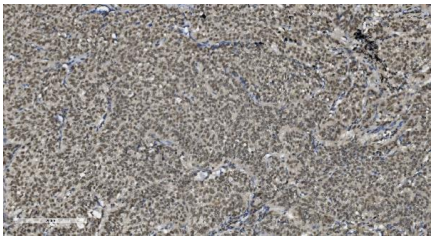


Figure 6. IHC analysis of U2AF65/U2AF2 using anti U2AF65/U2AF2 antibody (M03639-1).
U2AF65/U2AF2 was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-U2AF65/U2AF2 Antibody (M03639-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

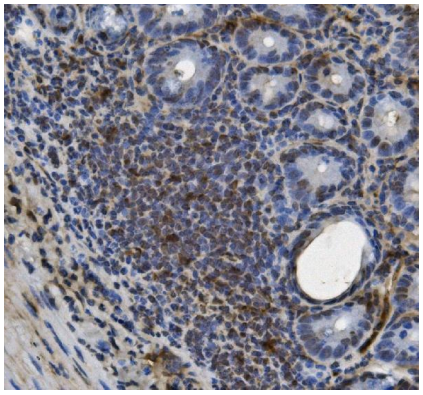


Figure 7. IHC analysis of U2AF65/U2AF2 using anti U2AF65/U2AF2 antibody (M03639-1).
U2AF65/U2AF2 was detected in paraffin-embedded section of mouse intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-U2AF65/U2AF2 Antibody (M03639-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

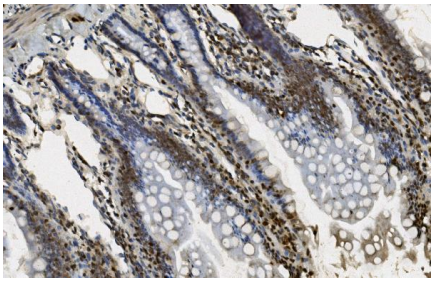


Figure 8. IHC analysis of U2AF65/U2AF2 using anti U2AF65/U2AF2 antibody (M03639-1).
U2AF65/U2AF2 was detected in paraffin-embedded section of rat intestine tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-U2AF65/U2AF2 Antibody (M03639-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

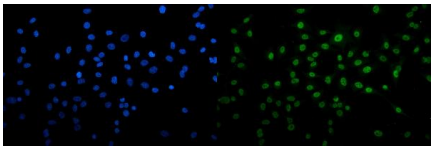


Figure 9. IF analysis of U2AF65/U2AF2 using anti-U2AF65/U2AF2 antibody (M03639-1).
U2AF65/U2AF2 was detected in immunocytochemical section of A549 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-U2AF65/U2AF2 Antibody (M03639-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) 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.

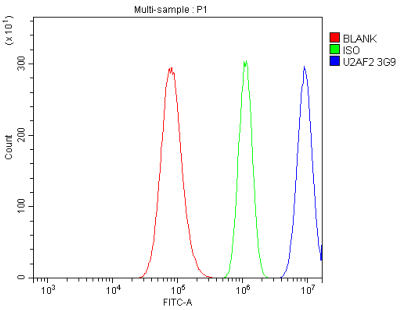


Figure 10. Flow Cytometry analysis of A549 cells using anti-U2AF65/U2AF2 antibody (M03639-1).
Overlay histogram showing A549 cells stained with M03639-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-U2AF65/U2AF2 Antibody (M03639-1, 1 µg/1x10^6 cells) 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.

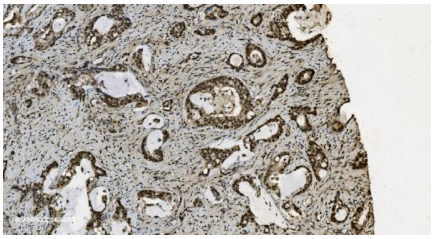


Figure 11. IHC analysis of U2AF65/U2AF2 using anti U2AF65/U2AF2 antibody (M03639-1).
U2AF65/U2AF2 was detected in paraffin-embedded section of human gallbladder adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-U2AF65/U2AF2 Antibody (M03639-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

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