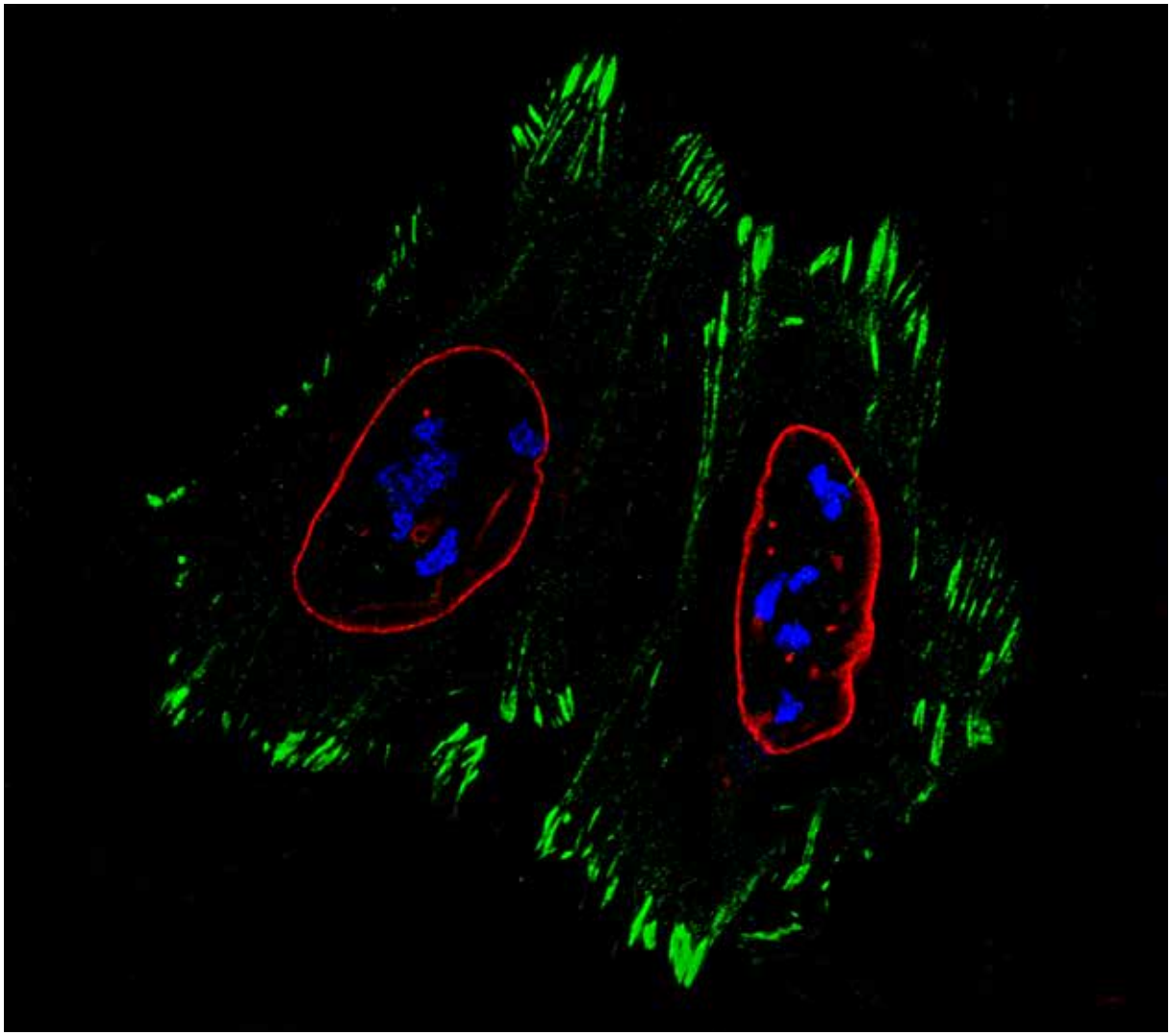


Organelle Marker Panel

PrecisA MONOCLONALS™
DESIGNED BY RESEARCHERS FOR RESEARCHERS

ATLAS ANTIBODIES

atlasantibodies.com

Organelle Markers

Organelle Markers in subcellular localization studies

Subcellular localization studies are important for mapping and characterizing proteins and thus for better understanding of the cellular functions of the proteins. By confocal microscopy analysis on human cell lines, spatial and temporal protein expression patterns can be visualized on a fine cellular and subcellular level.

In order to facilitate interpretation of the subcellular distribution of the protein targeted by a specific antibody, the cells may be stained with reference markers for different subcellular structures, organelles, within the cells. For example, in the Human Protein Atlas project, an antibody towards calreticulin was chosen as a reference marker for the Endoplasmic Reticulum (ER). This is illustrated in Figure 1.

For instance, for automated annotation of co-localization analysis, organelle specific markers are needed for every subcellular compartment to be analyzed.

Atlas Antibodies´ Organelle Marker Panel

In collaboration with the Human Protein Atlas project, a number of reference markers for different organelles have been developed. These includes 27 monoclonal antibodies targeting 15 different subcellular structures within the cell. These are presented in Figure 3 and Table 1.

The Organelle Markers of the panel have been selected based primarily on the specific target recognition over a number of commonly used human cell lines, such as A-431, U-251 MG, U-2 OS, HeLa and MCF-7. Other selection criteria of the markers included high signal to noise ratio, agreement of protein-RNA expression according to RNA Seq data, detection of band of expected target size in WB, as well as correlation of positivity to the results of other antibodies towards the same protein target.

These monoclonal Organelle Markers have been developed together as a panel under the stringent conditions of the PrecisA Monoclonal Antibodies, which guarantees a secured continuity and stable supply.

The majority of the Organelle Markers in this panel are also recommended for Western Blot (WB) and Immunohistochemistry (IHC) applications, as exemplified with Anti-TUFM in Figure 2.

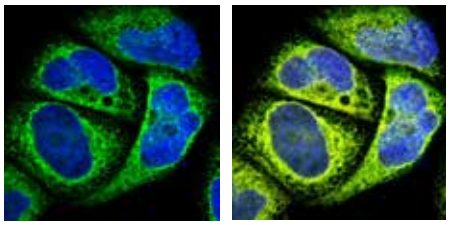


Figure 1.
Anti-HSP90B1 (HPA003901) staining is shown in green, nuclear reference DAPI in blue and the endoplasmic reticulum (ER) reference marker detecting calreticulin shown in yellow. The yellow signal overlaps with the green antibody signal, confirming ER-specificity.

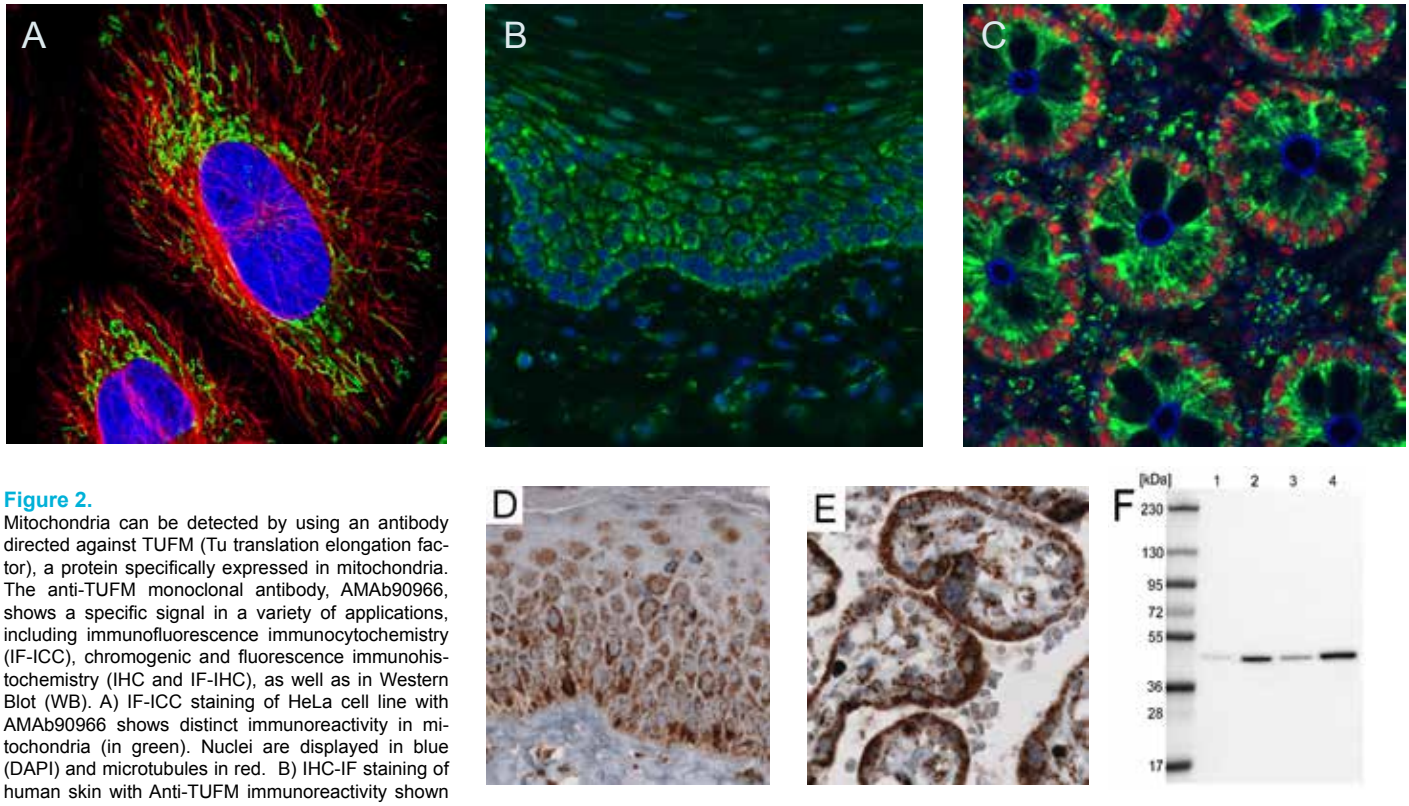
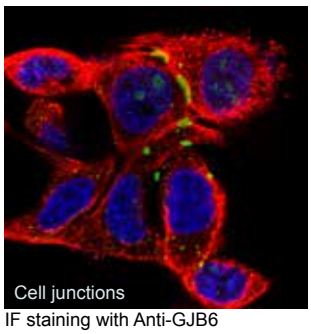


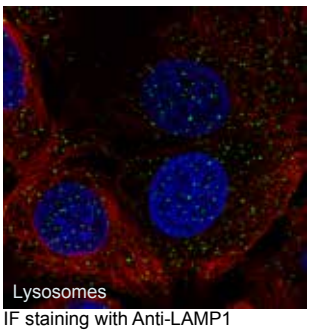
Figure 2.
Mitochondria can be detected by using an antibody directed against TUFM (Tu translation elongation factor), a protein specifically expressed in mitochondria. The anti-TUFM monoclonal antibody, AMAb90966, shows a specific signal in a variety of applications, including immunofluorescence immunocytochemistry (IF-ICC), chromogenic and fluorescence immunohistochemistry (IHC and IF-IHC), as well as in Western Blot (WB). A) IF-ICC staining of HeLa cell line with AMAb90966 shows distinct immunoreactivity in mitochondria (in green). Nuclei are displayed in blue (DAPI) and microtubules in red. B) IHC-IF staining of human skin with Anti-TUFM immunoreactivity shown in green and nuclei in blue. C) Multiplexed IHC-IF staining of human colon tissue with mitochondrial Anti-TUFM (AMAb90966, IgG1) immunoreactivity shown in green, plasma membranes in blue (Anti-EZR, AMAb90979, IgG2b) and nuclei in red (Anti-HNRNPC, AMAb91010, IgG2a). D) IHC staining of human skin and E) placenta. F) WB analysis of human cell lines U251 MG (1), A-431 (2), U-2 OS (3) and MCF-7 (4).



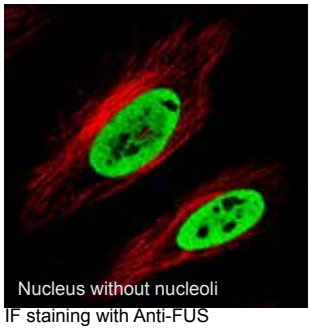
IF staining with Anti-GJB6 (AMAb91305) shows positivity in cell junctions.

IF staining with Anti-CDK5RAP2 (AMAb91163) shows positivity in centrosome.

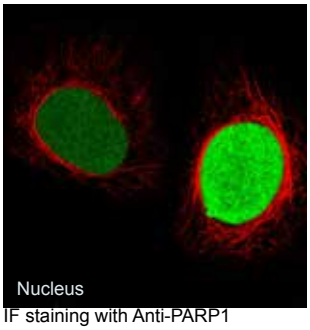
IF staining with Anti-DES (AMAb91303) shows positivity in intermediate filaments.



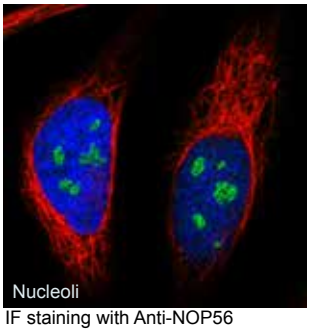
IF staining with Anti-LAMP1 (AMAb91170) shows positivity in lysosomes.



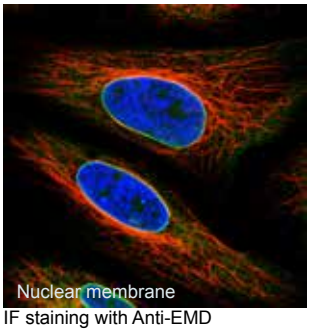
IF staining with Anti-FUS (AMAb90549) shows nuclear (without nucleoli) positivity.



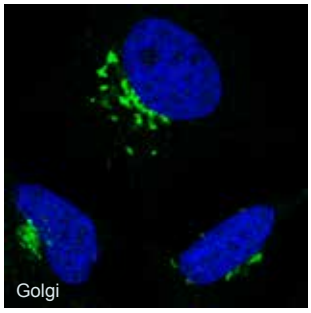
IF staining with Anti-PARP1 (AMAb90959) shows a cell cycle dependent nuclear (without nucleoli) pattern.



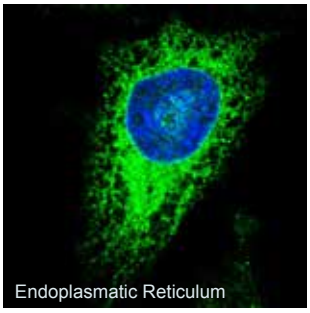
IF staining with Anti-NOP56 (AMAb91013) shows nucleoli-specific positivity.



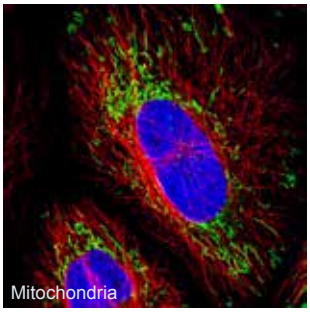
IF staining with Anti-EMD (AMAb90562) shows strong positivity in nuclear membrane, as well as weaker positivity in ER.



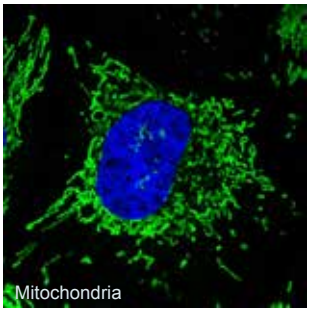
IF staining with Anti-GORASP2 (AMAb91016) shows positivity in Golgi apparatus.



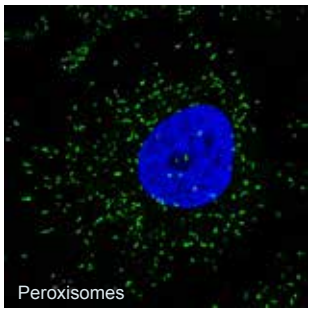
IF staining with Anti-PDIA3 (AMAb90988) shows distinct positivity of endoplasmic reticulum (ER).



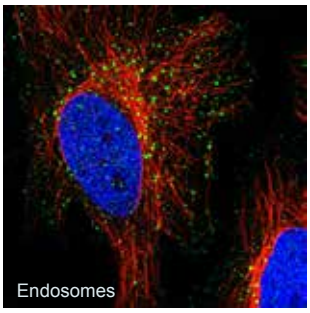
IF staining with Anti-TUFM (AMAb90966) shows specific mitochondrial staining.



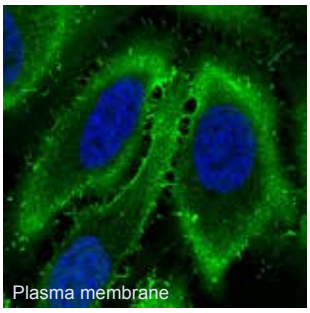
IF staining with Anti-CS (AMAb91005) shows distinct positivity in mitochondria.



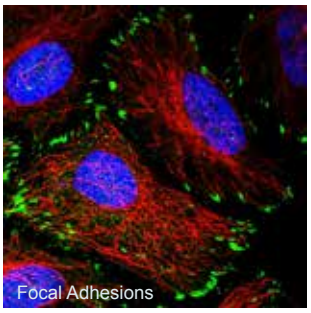
IF staining with Anti-ABCD3 (AMAb90995) shows positivity in peroxisomes.



IF staining with Anti-VPS26A (AMAb90967) shows distinct staining of endosomes.



IF staining with Anti-EZR (AMAb90976) shows positivity in plasma membrane.



IF staining with Anti-ZYX (AMAb90992) shows distinct staining of focal adhesions.

Figure 3
Immunofluorescence images showing 15 different subcellular organelles using PrecisA Monoclonals as markers with antibody staining shown in green. Microtubule- and nuclear probes are visualized in red and blue respectively (where available).

Table 1.
Description of the PrecisA Monoclonals included in the Organelle Marker panel.

Organelle	Target gene description	Product Name	Product Number	Validated Applications	Isotype
Cell junctions	Gap junction protein, beta 6, 30kda	Anti-GJB6	AMAb91305	ICC-IF	IgG2a
Centrosome	Cdk5 regulatory subunit associated protein 2	Anti-CDK5RAP2	AMAb91163	ICC-IF, IHC	IgG1
Centrosome	Centrosomal protein 350kda	Anti-CEP350	AMAb91164	ICC-IF, IHC	IgG1
Intermediate filaments	Desmin	Anti-DES	AMAb91303	ICC-IF, WB	IgG2a
Lysosomes	Lysosomal-associated membrane protein 1	Anti-LAMP1	AMAb91170	ICC-IF, IHC, WB	IgG2a
Nucleus (without nucleoli)	Fused in sarcoma	Anti-FUS	AMAb90549	ICC-IF, IHC, WB	IgG1
Nucleus (without nucleoli)	Heterogeneous nuclear ribonucleoprotein C (C1/C2)	Anti-HNRNPC	AMAb91010	ICC-IF, IHC, WB	IgG2a
Nucleus (without nucleoli)	Heterogeneous nuclear ribonucleoprotein C (C1/C2)	Anti-HNRNPC	AMAb91012	ICC-IF, IHC, WB	IgG1
Nucleus (without nucleoli)	Anillin, actin binding protein	Anti-ANLN	AMAb90662	ICC-IF, IHC, WB	IgG1
Nucleus	Poly (ADP-ribose) polymerase 1	Anti-PARP1	AMAb90959	ICC-IF, IHC, WB	IgG1
Nucleoli	MKI67 (FHA domain) interacting nucleolar phosphoprotein	Anti-MKI67IP	AMAb90961	ICC-IF, IHC	IgG2a
Nucleoli	NOP56 ribonucleoprotein homolog	Anti-NOP56	AMAb91013	ICC-IF, IHC, WB	IgG1
Nuclear membrane	Emerin	Anti-EMD	AMAb90562	ICC-IF, IHC, WB	IgG1
Nuclear membrane	Lamin b1	Anti-LMNB1	AMAb91251	ICC-IF, IHC	IgG1
Golgi apparatus	Golgi reassembly stacking protein 2	Anti-GORASP2	AMAb91016	ICC-IF, IHC, WB	IgG2b
Endoplasmic reticulum	Protein disulfide isomerase family A	Anti-PDIA3	AMAb90988	ICC-IF, IHC, WB	IgG1
Endoplasmic reticulum	Heat shock protein 90kda beta (grp94), member 1	Anti-HSP90B1	AMAb91019	ICC-IF, IHC, WB	IgG2b
Mitochondria	Tu translation elongation factor, mitochondrial	Anti-TUFM	AMAb90964	ICC-IF, IHC, WB	IgG1
Mitochondria	Tu translation elongation factor, mitochondrial	Anti-TUFM	AMAb90965	ICC-IF, IHC, WB	IgG2a
Mitochondria	Tu translation elongation factor, mitochondrial	Anti-TUFM	AMAb90966	ICC-IF, IHC, WB	IgG1λ
Mitochondria	Citrate synthase	Anti-CS	AMAb91005	ICC-IF, IHC, WB	IgG1
Mitochondria	Citrate synthase	Anti-CS	AMAb91007	ICC-IF, IHC, WB	IgG1
Mitochondria	Citrate synthase	Anti-CS	AMAb91009	ICC-IF, IHC, WB	IgG1
Peroxisomes	ATP-binding cassette, sub-family D (ALD), member 3	Anti-ABCD3	AMAb90995	ICC-IF, IHC	IgG1
Endosomes	Vacuolar protein sorting 26 homolog A	Anti-VPS26A	AMAb90967	ICC-IF, IHC, WB	IgG1
Plasma membrane	Ezrin	Anti-EZR	AMAb90976	ICC-IF, IHC, WB	IgG1
Focal adhesions	Zyxin	Anti-ZYX	AMAb90992	ICC-IF, IHC	IgG2b

Cover image:
Multiplexed ICC-IF staining of HeLa cells illustrating nuclear membrane in red (Lamin B1), nucleoli in blue (MKI67IP) and focal adhesions in green (Zyxin). The ICC-IF staining was performed using primary antibodies of different isotypes: Anti-LMNB1 AMAb91251 (IgG1), and Anti-MKI67IP AMAb90961 (IgG2a) and Anti-Zyx AMAb90992 (IgG2b). Alexa Fluor® 647-, 594- and 488-labelled isotype-specific secondary antibodies (ThermoFisher Scientific) were used for visualization.

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