Technical Data Sheet

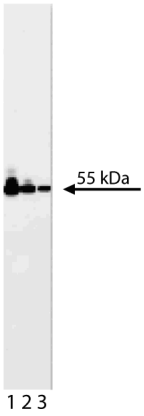
Purified Mouse Anti-ATP Synthase β

Product Information

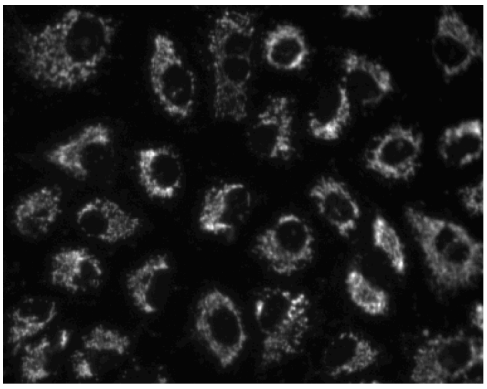
Material Number:	**612518**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	10/ATP
Immunogen:	Human ATP Synthase β aa. 428-539
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Chicken, Dog, Mouse, Rat
Target MW:	55 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

ATP synthase is a large enzyme complex that uses an electrochemical H+ or Na+ gradient to synthesize ATP from ADP and Pi, providing the organism with the ATP needed for energy. The complex consists of two major units, F0 and F1. F0 is embedded in the inner membrane of the mitochondria and, due to its hydrophobic nature, translocates protons across this membrane. F1 is the catalytic portion in the matrix region of the mitochondria and is comprised of α, β, γ, δ, and ε subunits at a 3:3:1:1:1 ratio. The β subunit is synthesized in the nuclear genome,transported to the mitochondria, and assembled with the other subunits. It is encoded by a single copy gene, is ubiquitously expressed and highly conserved among species. ATP synthase β contains an Ets domain binding site, which is a main site for promoter activity. Ets proteins contain domains that are involved in transcriptional activation, protein-protein interactions, and intramolecular repression of DNA binding. This site acts as a target for transcriptional control by the Ets family of transcription factors. Thus, ATP synthase β is involved in the synthesis of ATP and is controlled in part by ETS family proteins.



Western blot analysis of ATP Synthase β on a Jurkat cell lysate. *Lane 1: 1:10,000, Lane 2: 1:20,000, Lane 3: 1:40,000 dilution of the Mouse Anti-ATP Synthase β antibody.*



Immunofluorescent staining of A549 (ATCCCCL-185) cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~10,000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the Mouse Anti-ATP Synthase β antibody. The second step reagent was FITC goat anti- mouse Ig (Cat. No. 554001). The image was taken on a BD Pathway™ 855 bioimaging system using a 20x objective. This antibody also stains HeLa (ATCC CCL-2) and U-2 OS(ATCC HTB-96) cells and can be used with either fix/perm protocol (see Recommended Assay Procedure).*

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Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/monoclonal_anti.jsp

Suggested Companion Products

Catalog Number	Name	Size	Clone
611451	Jurkat Cell Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
3. Triton is a trademark of the Dow Chemical Company.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Lee JH, Garboczi DN, Thomas PJ, Pedersen PL. Mitochondrial ATP synthase. cDNA cloning, amino acid sequence, overexpression, and properties of the rat liver alpha subunit. *J Biol Chem.* 1990; 265(8):4664-4669. (Biology)

Ohta S, Tomura H, Matsuda K, Kagawa Y. Gene structure of the human mitochondrial adenosine triphosphate synthase beta subunit. *J Biol Chem.* 1988; 263(23):11257-11262. (Biology)

Villena JA, Martin I, Vinas O, et al. ETS transcription factors regulate the expression of the gene for the human mitochondrial ATP synthase beta-subunit. *J Biol Chem.* 1994; 269(51):32649-32654. (Biology)