Data Sheet (Cat.No.TMAB-00870)

TargetMol

Anti-HMGB1 Polyclonal Antibody

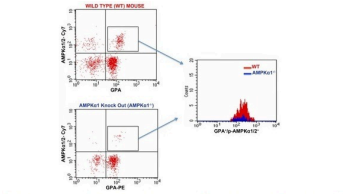
Product Details

Ig Type:	IgG
Reactivity:	Human,Rat (predicted:Mouse,Cow)
Molecular Weight:	Theoretical: 25 kDa.
Purification:	Protein A purified

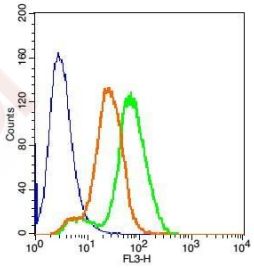
Applications

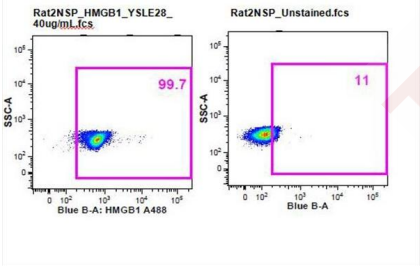
Verified Activity:

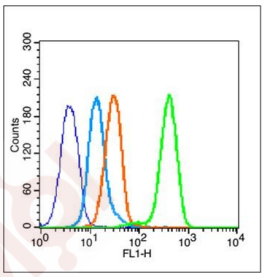
1. The figure annotation:
The blue histogram is unstained cells.
The Orange histogram is cells stained with Rabbit IgG/FITC
The green histogram is cells stained with Rabbit Anti-HMGB1/FITC Conjugated antibody (TMAB-00870-FITC).
Controls
Positive control: HepG 2 cells
Isotype control: Cell lines treated with Rabbit IgG/FITC. instead of the primary antibody to confirm that primary antibody binding is specific. 5 μgin 100 μL 1 X PBS containing 0.5% BSA.
2. Positive control: HepG2 cells
Concebtration: 5 μg/10^6 cells
Incubation conditions: Avoid light , 30 minutes on the ice.
3. Rat splenocytes stained with Anti-HMGB1 Polyclonal Antibody, A488 Conjugated (TMAB-00870-A488) at 1:50.
4. Blank control (blue line): MCF7 (blue).
Primary Antibody (green line): Rabbit Anti-HMGB1 antibody (TMAB-00870)
Dilution: 1 μg/10^6 cells;
Isotype Control Antibody (orange line): Rabbit IgG.
Secondary Antibody (white blue line): Goat anti-rabbit IgG-FITC
Dilution: 1 μg/test.
Protocol
The cells were fixed with 70% ethanol (Overnight at 4°C) and then permeabilized with 90% ice-cold methanol for 30 min on ice. Cells stained with Primary Antibody for 30 min at room temperature. The cells were then incubated in 1 X PBS/2% BSA/10% goat serum to block non-specific protein-protein interactions followed by the antibody for 15 min at room temperature. The secondary antibody used for 40 min at room temperature.
5. Blank control: HL-60. Primary Antibody (green line): Rabbit Anti-HMGB1 antibody (TMAB-00870)
Dilution: 1 μg/10^6 cells;
Isotype Control Antibody (orange line): Rabbit IgG.
Secondary Antibody: Goat anti-rabbit IgG-AF488
Dilution: 1 μg/test.
Protocol
The cells were fixed with 4% PFA (10 min at room temperature) and then permeabilized with 90% ice-cold methanol for 20 min at-20°C. The cells were then incubated in 5% BSA to block non-specific protein-protein interactions for 30 min at room temperature. Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature.

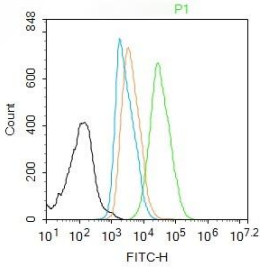


AMPK, a hetero-trimeric enzyme, is the master-regulator of cellular energetics and metabolism. AMPKα with two isoforms (AMPKα1 and AMPKα2) is the catalytic unit of AMPK. AMPKα1 is a predominant isoform in endothelial cells, immune cells as well as circulating blood cells. RBCs primarily (70 ~ 90%) express AMPKα1. Genetic deletion of AMPKα1 in mouse causes loss in RBCs deformability index (that is increased RBCs rigidity) and severe splenomegaly. In humans diabetics have poor AMPK-activity and erythrocytic DI-values. Therefore, we analyzed RBCs for AMPK-activity via FACS-analysis of AMPKα1 phosphorylation with RBC-associated marker GPA. Whole blood samples were stained with fluorochrome-conjugated antibodies as shown above and analyzed using a four-color flow cytometer (FACS Calibur, BD Biosciences, San Diego, CA) and CellQuest software. Very mild p-AMPKα present in the AMPKα1 KO mouse can be seen due to presence of low level of AMPKα2 in RBCs.









Application:	FCM
Recommended	FCM: 1μg/Test

Properties

Stability & Storage:	Store at -20°C or -80°C for 12 months. Avoid repeated freeze-thaw cycles.
Shipping:	Shipping with blue ice.

Antigen Details

Immunogen:	KLH conjugated synthetic peptide: human HMGB1
Antigen Species:	Human
Gene ID:	3146
Uniprot ID:	P09429
Synonyms:	HMG1;HMGB1;HMG3;HMG-1;SBP-1
Biology Area:	HMGs,HMG Box,SARS Coronavirus,hmgb1

Research Background

High Mobility Group Box-1 (HMGB1) is a cytokine implicated in the pathogenesis of rheumatoid arthritis (RA) and other inflammatory diseases. The cholinergic anti-inflammatory pathway, a vagus nerve dependent mechanism, inhibits HMGB1 release in experimental disease models

Inhibitor · Natural Compounds · Compound Libraries · Recombinant Proteins

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