

## Exosomal Marker CD63 Antibody Set Kit

Cat. Number: hEXOWBCD63-5

Stable for at least 6 MONTHS  
from the date of shipment.

For Research Use Only. Not For Use In Diagnostic Procedures.

### Products Information

Products Included	Quantity	Mol. Wt.	Storage conditions	Isotype	Dilution
Anti-human CD63 mouse mAb	25 $\mu$ L 1.0mg/mL	56kD	2-8 $^{\circ}$ C <b>DO NOT FREEZE</b>	Mouse IgG1, $\kappa$	<b>1:1,500</b> (1:500-1:2,000)
Anti-mouse IgG,HRP-linked mAb	25 $\mu$ L	-----	2-8 $^{\circ}$ C <b>DO NOT FREEZE</b>	Rabbit IgG	<b>1:3,000</b> (1:2,000-1:10,000)
5 $\times$ SDS Sample Buffer I	250 $\mu$ L	-----	-20 $^{\circ}$ C	-----	-----
Exosome Positive Control	100 $\mu$ L	-----	-20 $^{\circ}$ C	-----	-----

### Product description

The Exosomal Marker (CD63) Antibody Set Kit provides an economical means to evaluate the presence of exosomal markers. The kit includes enough primary antibody to perform at least **5** western blot experiments for each target. The secondary antibody that is included in this kit has been optimized to enhance the signal-to-noise ratio. We do not recommend using other secondary antibodies with the primary antibodies at this time.

### Background

Exosomes are small membrane-bound vesicles that in recent years have emerged as

important molecules for inter-cellular communication. Exosomes are produced during both normal and patho/physiological conditions, and cancer cells have been shown to secrete exosomes in greater amounts than normal cells.

Anti-CD63 recognizes an extracellular fragment of CD63, a 56-kilodalton (kDa), type III lysosomal glycoprotein, belonging to the tetraspanin family. CD63 is expressed by granulocytes, platelets, T-cells, monocytes/macrophages, endothelial cells. CD63 protein is also expressed in exosome membrane. CD63 protein is a canonical exosome marker and currently used to characterize exosome populations from a variety of body fluids. **These glycosylations can affect the size at which the marker proteins appear on a Western blot and bands may appear at several different sizes.**

## Western Immunoblotting Protocol

### A. Solutions and Reagents

1. 20X Phosphate Buffered Saline (PBS).
2. 10X Tris Buffered Saline (TBS).
3. 10X Tris-Glycine SDS Running Buffer.
4. 10X Tris-Glycine Transfer Buffer.
5. 10X Tris Buffered Saline with Tween® 20 (TBST).
6. Blocking Buffer: 1X TBST with 4% w/v nonfat dry milk.
7. Wash Buffer: 1X TBST
10. Primary Antibody Dilution Buffer: 1X TBST with 4% BSA
11. Blotting Membrane : This protocol has been optimized for nitrocellulose membranes. Pore size 0.45µm is generally recommended.
12. Secondary Antibody Conjugated to HRP: anti-mouse IgG,HRP-linked mAb.
13. Detection Reagent: Clarity™Western ECL Substrate(BIO-RAD Cat.#170-5056).

### B. Protein Blotting

1. Lyse exosome samples by adding **5X SDS sample buffer I** (for 20µL sample,add 4µL 5X SDS sample buffer),mix well and **99°C for 10min**.
2. Microcentrifuge for 5 min. Load 20µL onto SDS-PAGE gel (10 cm x 10cm).

**NOTE:** Loading of prestained molecular weight markers to verify electrotransfer and

determine molecular weights are recommended.

3. Electrotransfer to nitrocellulose membrane.

### C. Membrane Blocking and Antibody Incubation.

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane, for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 8min each with 15 ml of TBST. Incubate membrane and primary antibody (at the appropriate dilution, **1:1,500**(1:500-1:2,000)) in **7.5** ml primary antibody dilution buffer with gentle agitation at room temperature for 2h or overnight at 4°C.
4. Wash three times for 5 min each with 15 ml of TBST. Incubate membrane with the Anti-mouse IgG,HRP-linked mAb (**1:3,000**(1:2,000-1:10,000)) and in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
5. Wash three times for 5 min each with 15 ml of TBST.
6. Proceed with detection (Section D).

### D. Detection of Proteins

1. Incubate membrane with 10 ml Clarity™ Western ECL Substrate (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film or Chemiluminescence imager .

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.

## Related Products

Exosome Isolation & Purification	
Exosome Extraction & Purification Kits (for blood serum/plasma)	EXORG10SP-1/ EXORG30SP-1/
Exosome Concentration Kits (for cell culture media/urine)	EXOCon5-10/ EXOCon10-10
Exosome Capture and Isolation Kits (for cell culture media/urine)	EXOMCUCD9-10
	EXOMCUCD63-10
	EXOMCUCD81-10

Exo-Antibody	
Purified Anti-human Alix Antibody	RGAB100-50/RGAB100-100
Purified Anti-human CD9 Antibody	RGAB101-50/RGAB101-100
Anti-human CD9 Ab Biotin Conjugated	RGAB102-50/RGAB102-100
Purified Anti-human CD63 Antibody	RGAB103-50/RGAB103-100
Anti-human CD63 Ab Biotin Conjugated	RGAB104-50/RGAB104-100
Purified Anti-human CD81 Antibody	RGAB105-50/RGAB105-100
Anti-human CD81 Ab Biotin Conjugated	RGAB106-50/RGAB106-100
Purified Anti-human TSG101 Antibody	RGAB107-50/RGAB107-100
Purified Anti-human PD-L1 Antibody	RGAB108-50/RGAB108-100
Anti-human PD-L1 Ab Biotin Conjugated	RGAB109-50/RGAB109-100
Purified Anti-human EpCAM Antibody	RGAB110-50/RGAB110-100
Anti-human EpCAM Ab Biotin Conjugated	RGAB111-50/RGAB111-100
Other Exosomal Markers Ab Set	
Exosomal Marker CD9 Antibody Set Kit	hEXOWBCD9-5
Exosomal Marker CD81 Antibody Set Kit	hEXOWBCD81-5
Exosomal Marker Alix Antibody Set Kit	hEXOWBALix-5
Exosomal Marker Tsg101 Antibody Set Kit	hEXOWBTsg101-5

## Technical Support

For more information about our products and to download manuals, please visit our web site: <http://www.rengenbio.com>

For additional information or technical assistance, please call or email us.

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