Exosomal Marker Tsg101 Antibody Set Kit

Cat. Number: hEXOWBTsg101-5

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 Tsg101 mouse mAb	250μL 0.2mg/mL	45kD	2-8℃ DO NOT FREEZE	Mouse IgG_{2a}, κ	1:200 (1:100-1:1,000)
Anti-mouse IgG,HRP-linked mAb	25μL	-----	2-8℃ DO NOT FREEZE	Rabbit IgG	1:3,000 (1:2,000-1:10,000)
5×SDS Sample Buffer II	250 μL	-----	-20℃	-----	-----
Exosome Positive Control	100 μL	-----	-20℃	------	-----

Product description

The Exosomal Marker (Tsg101) 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.

TSG101, a 46 kDa protein, is the product of a recently identifed **Tumor Susceptibility Gene** whose inactivation in mouse fibroblasts results in cell transformation and the ability of those cells to form tumors in nude mice. TSG101 is highly expressed internally to EVs and it is considered one of the common markers for exosome detection.

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 II** (for 20μL sample,add 4μL **5X SDS sample buffer II**),mix well and 99℃for 10min.

2. Microcentrifuge for 5 min. Load 20μL onto SDS-PAGE gel (10 cm x 10 cm).

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^2) 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,1:200 (1:100-1:1,000)) in 10 ml primary antibody dilution buffer with gentle agitation at room temperature for 2h or overnight at 4℃.
4. Wash three times for 5 min each with 15 ml of TBST.
5. 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.
6. Wash three times for 5 min each with 15 ml of TBST.
7. 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-humanEpCAMAntibody	RGAB110-50/RGAB110-100
Anti-human EpCAM Ab Biotin Conjugated	RGAB111-50/RGAB111-100
Other Exosomal Markers Ab Set	
Exosomal Marker CD63 Antibody Set Kit	hEXOWBCD63-5
Exosomal Marker CD81 Antibody Set Kit	hEXOWBCD81-5
Exosomal Marker CD9 Antibody Set Kit	hEXOWBCD9-5
Exosomal Marker Alix Antibody Set Kit	hEXOWBAlix-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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