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MONOCLONAL ANTIBODY

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Catalog No. LKG-M002

Anti Mouse CD44 v10-e16 [Clone : RM1]

BACKGROUND

CD44 is a single-pass type I transmembrane protein and functions as a cellular adhesion molecule for hyaluronic acid, a major component of the extracellular matrix. It exists in numerous isoforms that are generated through alternative splicing of CD44 precursor mRNA. Whereas the standard isoform of CD44 (CD44 s) is expressed predominantly in hematopoietic cells and normal epithelial cell subsets, CD44 v (variant) isoforms, which contain additional insertions in the membrane-proximal extracellular region, are highly expressed in epithelial-type carcinomas[5]. Moreover, CD44 is reported as cell surface marker for **cancer stem cells (CSCs)** derived from solid tumors including breast, prostate, colon, head and neck and pancreatic cancer. Expression of CD44, especially variant isoforms (CD44 v8-10), contributes to reactive oxygen species (ROS) defense through upregulation of the synthesis of reduced glutathione (GSH), the primary intracellular antioxidant. CD44v8-10 interacts with and stabilizes xCT, a subunit of the cystine-glutamate transporter xc(-), and thereby promotes cystine uptake for GSH synthesis. The ability to avoid the consequences of exposure to high levels of ROS is required for cancer cell survival and propagation *in vivo*. CSCs, in which defense against ROS is enhanced by CD44v8-10 are thus thought to drive tumor growth, chemoresistance and metastasis[1-4].

Clone RM1, is a monoclonal antibody specific for **mouse CD44 v10-e16** can be used for FCM assay, and importantly, for the enrichment of CSCs using a cell sorter. RM1 can be applied towards understanding a variety of molecular mechanisms for cancer stem cells using *in vitro* cell-based assays such as "*in vitro* sphere formation assay" and "*in vivo* lung metastasis assay".

Product type	Primary antibody
Immunogen	Mouse CD44 v8-10 transfected cell
Rased in	Rat
Myeloma	X63-Ag8-653
Clone number	RM1
Isotype	IgG2a
Source	Ascites
Purification	Affinity purified by Protein G
Buffer	Phosphate buffered saline (PBS)* *NOTE: PBS doesn't contain preservative. Preservative is added based on the research purpose.
Concentration	0.5 mg / mL
Volume	200 uL (100 ug)
Label	Unlabeled
Specificity	Mouse CD44 v10-e16
Cross reactivity	Mouse. Other species is not tested.
Storage	Store cold (2 to 8 °C)

Application notes	・ **Flow cytomerty**: 1-10μg/mL
Recommended dilutions	Other applications have not been tested. Optimal dilutions/concentrations should be determined by the end user. Detailed procedure is provided in the following **PROTOCOLS.**

References

1) Nagano O., *et al.*, Oncogene. 2013 Jan 21., 1-8. PMID:23334333

2) Ishimoto T., *et al.*, Cancer Cell. 2011 Mar 8;19(3):387-400. PMID : 21397861

3) Yae T., *et al.*, Nat Commun. 2012 Jun 6;3:883. PMID: 22673910

4) Tsugawa H., *et al.*, Cell Host Microbe. 2012 Dec 13;12(6):764-77. PMID: 23245321

5) Tanabe KK., *et al.*, Lancet. 1993 Mar 20;341(8847):725-6. PMID: 8095628

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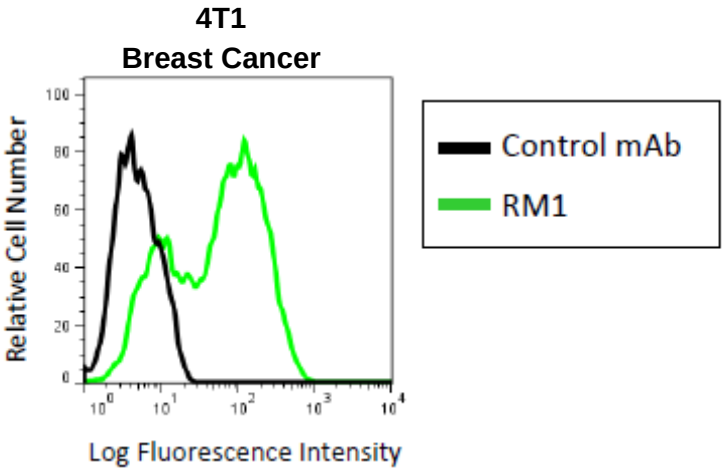

Fig.1 **Flow cytometry analysis** of CD44 v in **Mouse Breast cancer cell line 4T1** with anti-CD44 v10-e16 (RM1, 3µg/mL) antibody and PE-labeled anti Rat IgG antibody.

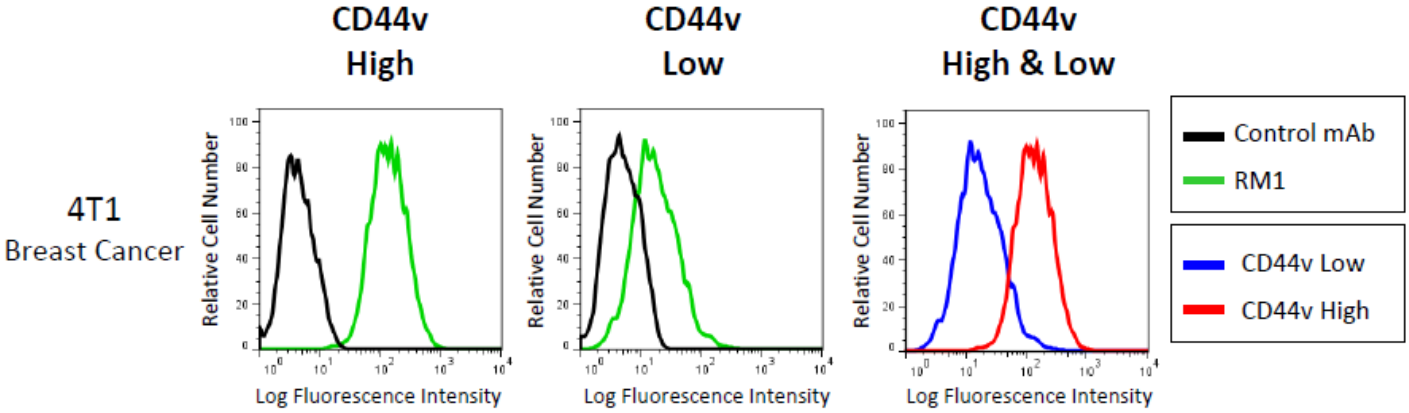

Fig.2 **Flow cytometry Cell Sorting** of CD44 v expression level in **Mouse Breast cancer cell line 4T1** with anti-CD44 v10-e16 (RM1) antibody and PE-labeled anti Rat IgG antibody.
Two kinds of subpopulations "CD44v^{+}(High) and CD44v^{-}(Low)" were isolated.

PROTOCOLS:

Flow cytometry protocol (Cell Analysis)

A. Cell Preparation
1. Remove cells from incubator.
2. Discard culture medium.
3. Briefly rinse the cell layer with PBS.
4. Add 0.25% trypsin-EDTA solution to dish. Return the dish to the incubator and incubate for 2-10 minutes or until cells are detached.
5. Resuspend cells in complete growth medium to inactivate the trypsin.

B. Staining
1. Aliquot $1x10^5$ cells into each assay tube.
2. Add 150 µl 0.2 % BSA in PBS to each tube and rinse by centrifugation.
3. Add 50 µl diluted primary antibody (3 µg/ml RM1 in 0.2 % BSA in PBS) to the assay tubes.
4. Incubate 45 minutes at 4 °C.
5. Add 100 µl 0.1 % BSA in PBS to each tube and wash by centrifugation.
6. Wash two times in 150 µl 0.1 % BSA in PBS by centrifugation.
7. Resuspend cells in 50 µl PE-labeled secondary antibody solution (Jackson Immuno Research 712-116-153), diluted 1:200 in 0.1 % BSA in PBS.
8. Incubate 30 minutes at 4 °C in the dark.
9. Add 100 µl 0.1 % BSA in PBS to each tube and wash by centrifugation.
10. Wash two times in 150 µl 0.1 % BSA in PBS by centrifugation.
11. Resuspend cells in 100 µl PBS.
12. Add 100 µl Propidium Iodide (SIGMA, P4864), diluted 1:500 in PBS, to stain dead cells.
13. Analyze using flow cytometry

Flow cytometry protocol (Cell Sorting)

A. Cell Preparation
1. Prepare cultured cells for sorting based on the ratio of the CD44v expression cells.
2. Remove cells from incubator.
3. Discard culture medium.
4. Briefly rinse the cell layer with PBS.
5. Add 0.25% trypsin-EDTA solution to dish. Return the dish to the incubator and incubate for 2-10 minutes or until cells are detached.
6. Resuspend the cells in complete growth medium to inactivate the trypsin.

B. Staining (for $1x10^7$ cells)
1. Aliquot $1x10^7$ cells into 15ml tube.
2. Add 10 ml 0.2 % BSA in PBS to the tube and rinse by centrifugation.
3. Add 5 ml diluted primary antibody (3 µg/ml RM1 in 0.2 % BSA in PBS) to the tube.
4. Incubate with gentle agitation 45 minutes at 4 °C.
5. Wash three times in 10 ml 0.1 % BSA in PBS by centrifugation.
6. Resuspend cells in 5 ml PE-labeled secondary antibody solution (Jackson Immuno Research 712-116-153), diluted 1:200 in 0.1 % BSA in PBS.
7. Incubate 45 minutes at 4 °C in the dark.
8. Wash three times in 10 ml 0.1 % BSA in PBS by centrifugation.
9. Resuspend cells in 5 ml PBS.
10. Add 5 ml Propidium Iodide (SIGMA, P4864) diluted 1:500 in PBS, to stain dead cells.
11. Sort CD44v high and low expression cells using a cell sorter.
12. Wash the sorted cells in 5 ml complete growth medium (added antibiotic drug) three times by centrifugation.
13. Culture the sorted cells and scale up.
    - * Note the passage number and analyze the cell population periodically using flow cytometry.
14. If desired, sort the cells again, they would be high-enrichment.

RELATED PRODUCT:

Product Name	Clone	Application	Quantity	Maker	Cat#
Anti Human CD44 v9 Monoclonal Antibody	RV3	FCM / IHC / IF / WB / IP / ELISA	100 ug / 100 uL	CAC	LKG-M001
Anti Mouse CD44 v10-e16 Monoclonal Antibody	RM1	FCM	100 ug / 200 uL	CAC	LKG-M002

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