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Inspiration for Life Science

MONOCLONAL ANTIBODY

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Catalog No. LKG-M001, LKG-M003

Anti Human CD44 v9 [Clone : RV3]

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. CD44 v8-10 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 RV3, is a monoclonal antibody specific for **human CD44 v9** can be used for FCM, WB, IHC, ICC, IP, and ELISA assay, and importantly, for the enrichment of CSCs using a cell sorter. RV3 can be applied towards understanding a variety of molecular mechanisms and towards the development of new medicines against 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	Human CD44 v8-10 transfected cell
Rased in	Rat
Myeloma	X63-Ag8-653
Clone number	RV3
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	1 mg / mL
Volume	**Catalog No.LKG-M001: 100 UL (100 UG), Catalog No.LKG-M003: 50 UL (50 UG)**
Label	Unlabeled
Specificity	Human CD44 v9
Cross reactivity	Other species is not tested.
Storage	Store cold (2 to 8 °C)

Application notes
Recommended dilutions

- **Flow cytomerty**: 1-10µg/mL
- **Immunohistochemistry**: 0.2µg/mL (Paraffin section)
- **Immunofluorescence**: 3µg/mL
- **Western blotting**: 1µg/mL
- **Immunoprecipitation**: 10µg/mL
- **ELISA**: 5µg/mL

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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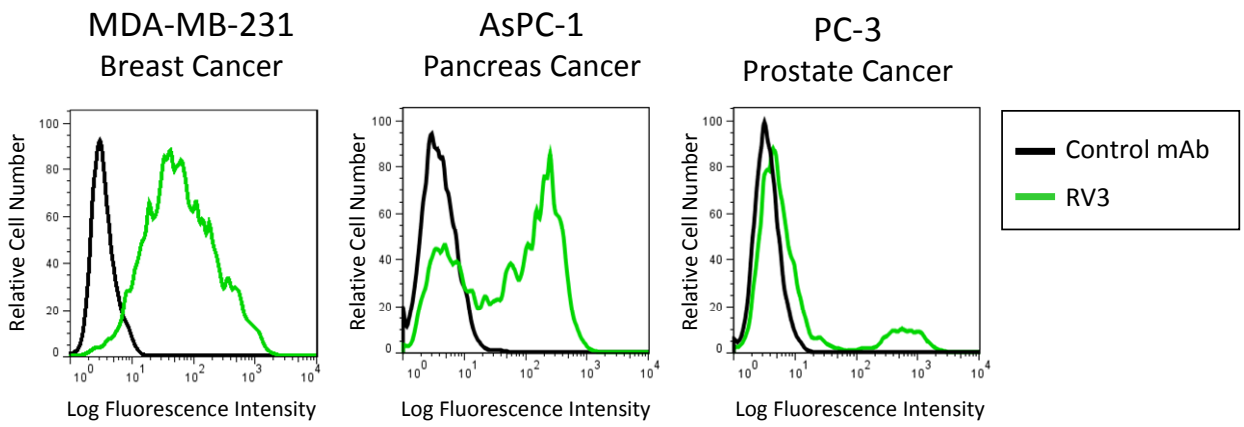

Fig.1 **Flow cytometry** analysis of CD44 v in Human cancer cell line with anti-CD44 v9 (RV3, 3µg/mL) antibody and PE-labeled anti Rat IgG antibody.

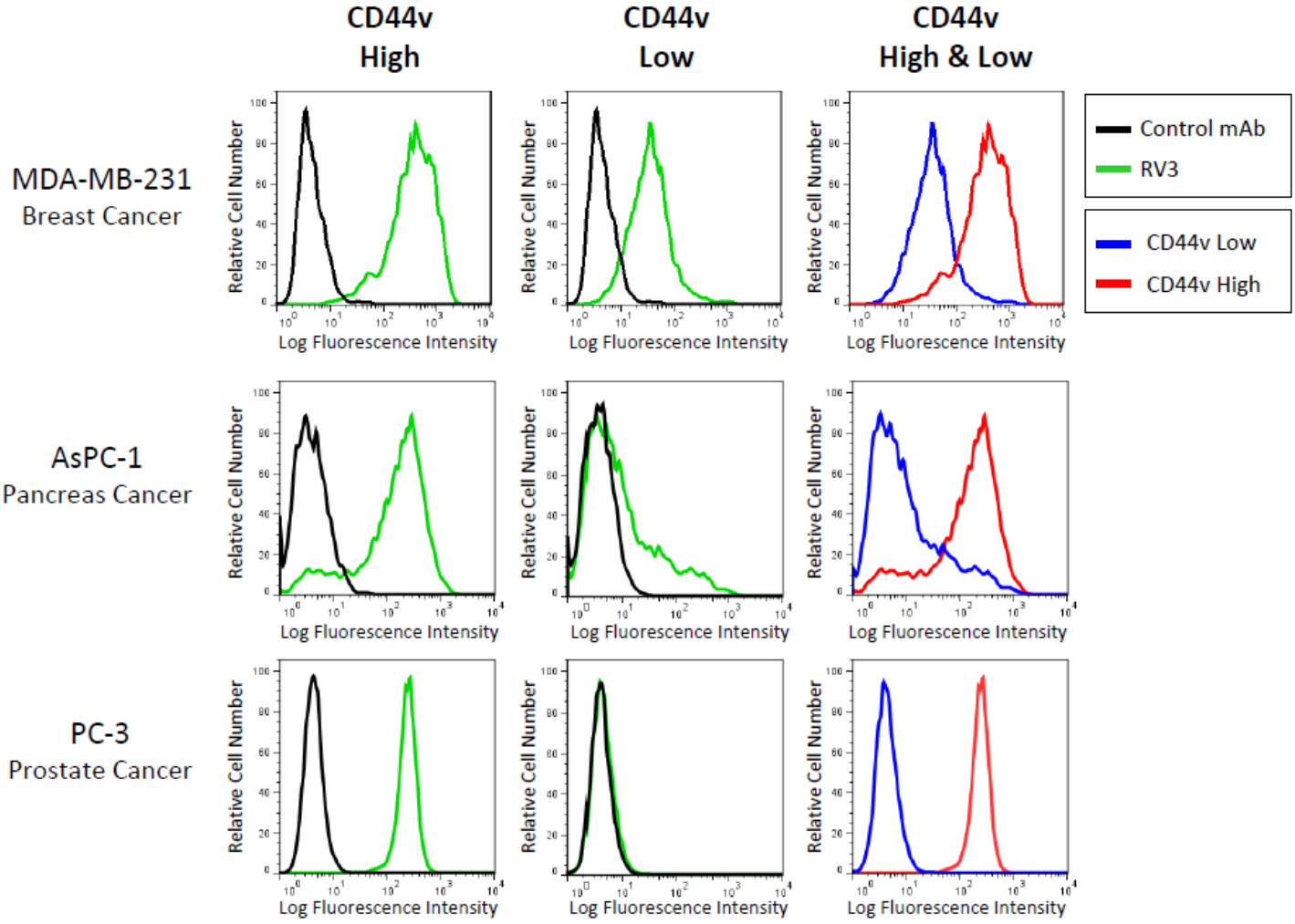

Fig.2 **Flow cytometry Cell Sorting** of CD44 v expression level in Human cancer cell line with anti-CD44 v9 (RV3) antibody and PE-labeled anti Rat IgG antibody.
Two kinds of subpopulations "$CD44v^+$(High) and $CD44v^-$(Low)" were isolated.

ANTIBODY CHARACTERIZATION

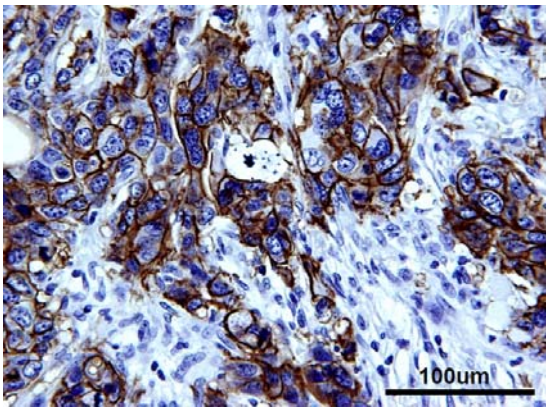

Fig.3 Immunohistochemistry staining Breast Invasive Ductal Carcinoma with anti-CD44 v9 antibody (clone RV3, 0.2µg/mL).

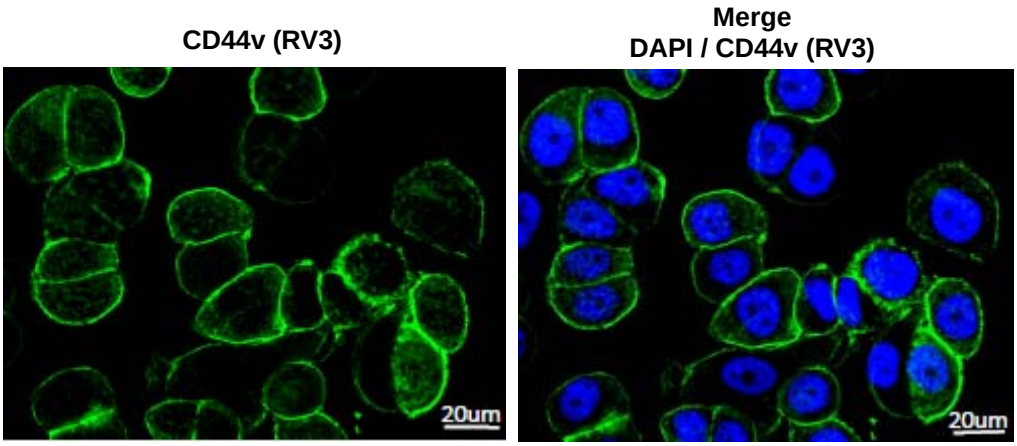

Fig.4 Immunofluorescence staining of CD44 v (green) in **MDA-MB-468 cells** with anti-CD44 v9 antibody (RV3, 3µg/mL).

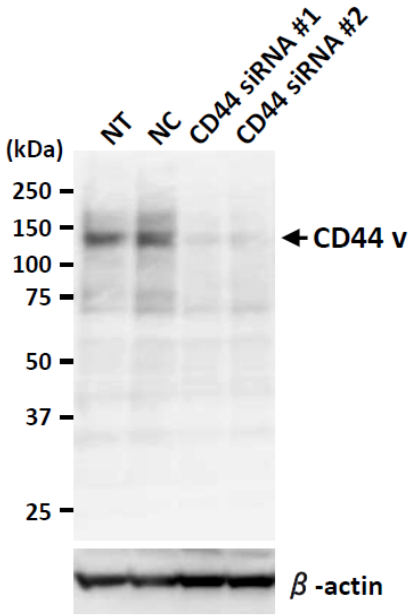

Fig.4 Western blot analysis of CD44 v in **BT20 cell lysate** with anti-CD44 v9 antibody(RV3, 1µg/mL). CD44 knockdown samples (CD44 siRNA #1, #2) were not detected.
NT: no treated **NC:** non-targeting siRNA treated sample

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 RV3 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 RV3 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.

Immunohistochemistry Protocol (Paraffin)

A. Deparaffinization / Rehydration

1. Deparaffinize/hydrate
    a. Incubate sections in xylene three times for 5 minutes each.
    b. Incubate sections in 100% ethanol for 10 seconds.
    c. Incubate sections in 90% ethanol for 10 seconds.
    d. Incubate sections in 70% ethanol for 10 seconds.
2. Wash sections in dH_2O for 5 minutes.

B. Antigen Unmasking

1. Immerse sections in 10 mM citrate buffer (pH 6.0) for 7 minutes at 100 °C in microwave.
2. Cool slides on bench top for 30 minutes.
3. Wash sections in TBS-T buffer for 5 minutes.

C.Staining

1. Incubate sections in 0.3% hydrogen peroxide in Methanol for 15 minutes to block endogenous peroxidase.
2. Wash sections in TBS-T buffer three times for 5 minutes each.
3. Cover sections with 300 µl blocking solution (10 % normal rabbit serum in TBS) for 30 minutes at room temperature.
4. Remove excess blocking solution and add 200 µl diluted primary antibody to each section (0.2µg/ml RV3 in 1.5 % normal rabbit serum in TBS). Incubate 1 hour at room temperature.
5. Remove antibody solution and wash sections in TBS-T buffer three times for 5 minutes each.
6. Add 200 µl biotinylated secondary antibody (Dako, E0468), diluted 1:200 in 1.5 % normal rabbit serum in TBS, to each section.
7. Incubate 30 minutes at room temperature.
8. Remove antibody solution and wash sections in TBS-T buffer three times for 5 minutes each.
9. Add 200 µl VECTASTAIN ABC Reagent (VECTOR LABORATORIES, PK-6100) to each section. Incubate 30 minutes at room temperature.
10. Wash sections in TBS-T buffer three times for 5 minutes each.
11. Add 200 µl peroxidase substrate reagent (PIERCE, 34065) to each section and incubate until desired stain intensity develops (about 1 minute).
12. Wash sections in TBS-T buffer three times for 5 minutes each.
13. Wash sections in dH_2O two times for 5 minutes each.
14. Counterstain sections in hematoxylin per manufacturer's instructions.
15. Dehydrate sections:
    a. Incubate sections in 70% ethanol for 10 seconds.
    b. Incubate sections in 90% ethanol for 10 seconds.
    c. Incubate sections in 100% ethanol for 10 seconds.
    d. Incubate sections in xylene three washes for 5 minutes each.
16. Mount coverslips.

Immunofluorescence Protocol

A. Cell Preparation

1. Grow cultured cells on Cell Chamber Slide.
2. Remove cells from incubator.
3. Discard medium and rinse briefly with PBS.
4. Fix in 4% paraformaldehyde in PBS for 15 minutes at room temperature.
5. Remove fixative and rinse three times in PBS for 3 minutes each.

B. Staining

1. Block sample in 3% BSA in PBS for 30 minutes at room temperature.
2. Remove excess blocking solution and apply diluted primary antibody (3 µg/ml RV3 in 0.2 % BSA in PBS).
3. Incubate 1 hour at room temperature.
4. Wash three times in PBS for 3 minutes each.
5. Apply Alexa Fluor 488 anti-rat IgG secondary antibody solution (Invitrogen, A11006), diluted 1:400 in 0.2 % BSA in PBS.
6. Incubate 1 hour at room temperature in the dark.
7. Wash three times in PBS for 3 minutes each.
8. Mount the coverslip using an anti-fade mounting reagent with DAPI (Invitrogen, S36939).
9. Seal slide by painting around edges of coverslip with nail polish.
10. Store slides flat at 4 °C protected from light until until examined.

Western blot protocol

1. Load 20 µl cell lysate samples (40µg/lane) onto SDS-PAGE gel (ATTO E-T10L).
2. Electrotransfer to PVDF membrane.
3. Block membrane in 5% Skim milk in PBS-T for 1 hour at room temperature.
4. Incubate membrane with 1 µg/ml RV3 primary antibody in Can Get Signal Solution I (TOYOBO, NKB201) with gentle agitation overnight at 4°C.
5. Wash membrane in PBS-T three times for 5 minutes each.
6. Incubate membrane with HRP-conjugated anti-rat IgG secondary antibody (GE Healthcare NA9350V), diluted 1:20000 in Can Get Signal Solution II (TOYOBO, NKB301), with gentle agitation 30 minutes at 37 °C.
7. Wash membrane in PBS-T three times for 5 minutes each.
8. Incubate membrane in detection mix (Thermo SuperSignal West Dura, 34075) with gentle agitation 1 minute at room temperature.
9. Detect using an imager.

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 Human CD44 v9 Monoclonal Antibody	RV3	FCM / IHC / IF / WB / IP / ELISA	50 ug / 50 uL	CAC	LKG-M003
Anti Mouse CD44 v10-e16 Monoclonal Antibody	RM1	FCM	100 ug / 200 uL	CAC	LKG-M002

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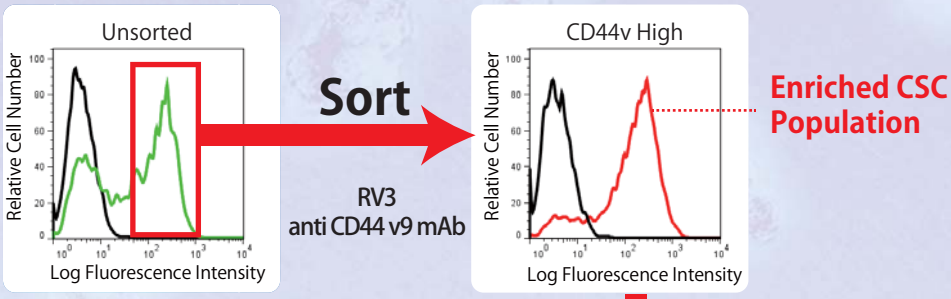
Cancer Stem Cell Enrichment!

Anti Human CD44 v9 mAb (clone RV3)
Anti Mouse CD44 v10-e16 mAb (clone RM1)

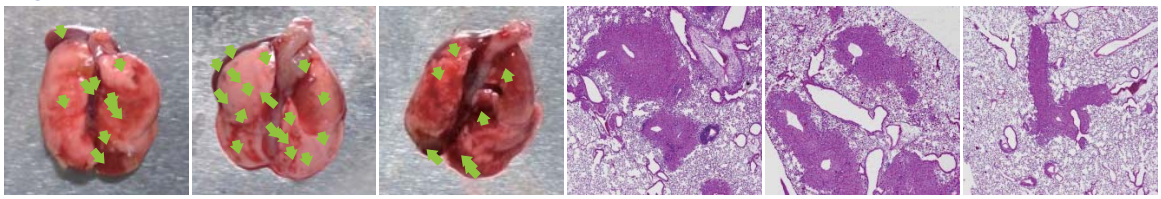
Powerful tools for *in vivo* CSC drug discovery and basic cancer research

Variant isoforms of CD44 (CD44v) are preferentially expressed on cancer stem cells (CSC). These highly specific CD44v monoclonal antibodies are well characterized and highly recommended for measuring CD44v expression by flow cytometry and for enrichment of CSC populations by cell sorting.

In vivo Lung metastasis assay study showing the high efficiency of CD44 v9-High cell populations sorted from the human pancreatic cancer cell line AsPC-1 to colonize mouse lung.



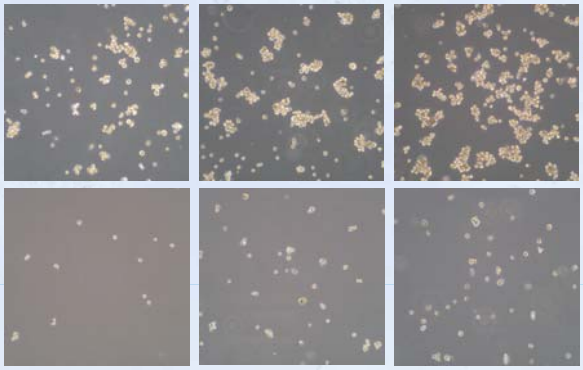
High metastasis formation in a CSC-dependent manner



Green arrows indicate readily observable metastatic colonies following injection of sorted CD44 v9-High cells into mice.
H&E staining is shown at right.

The high efficiency of metastasis (colony) formation by CD44 v9-High cells presents an opportunity to assay the effectiveness of new anti-CSC therapeutic strategies.

In vitro Sphere formation assays with CD44 v9-sorted human PC3 prostate cancer cells



Enriched CSC
High-CSC Population

Low-CSC Population

1,250 2,500 5,000 cells/well

Efficient sphere (tumor) formation by CD44 v9-High cells.

The high efficiency of sphere formation by CD44 v9-High cells presents an opportunity to assay the effectiveness of new anti-CSC therapeutic strategies.

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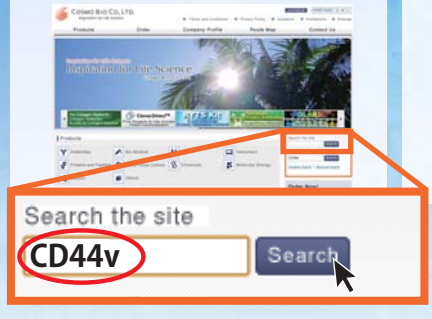


Order Information

Description	Host	Clone	Application	Cat. No.	Quantity
Anti Human CD44 v9	Rat	RV3	FCM/ IHC/ IF /WB/ IP/ ELISA	CAC-LKG-M001	100 µg
Anti Mouse CD44 v10-e16	Rat	RM1	FCM	CAC-LKG-M002	100 µg

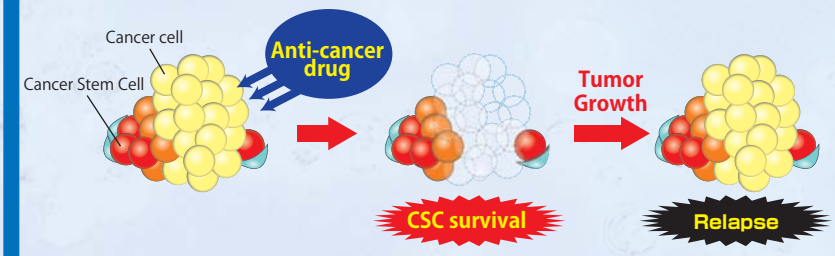
COSMO BIO CO., LTD.

Cancer Stem Cell Enrichment!

Anti Human CD44 v9 mAb (clone RV3) / Anti Mouse CD44 v10-e16 mAb (clone RM1)

Cancer Stem Cell [CSC] Characteristics

- Minor population in tumor : 0.1 - a few percent
- Self-renewing; infinite proliferative potential.
- Enhanced resistance to drugs, radiation, cell stress.
- Tumorigenic; give rise to other cell types in tumor.
- Associated with metastasis and relapse.



Metastasis and relapse are involved in more than 90% of all cancer deaths

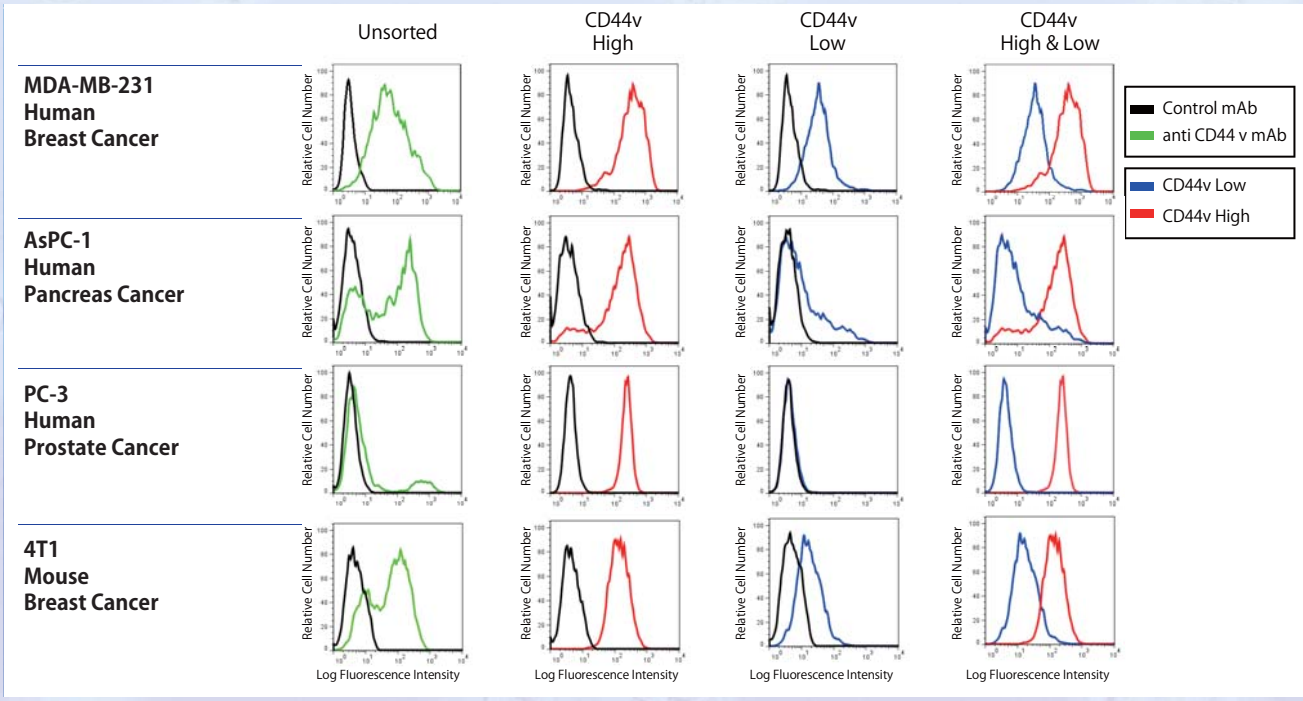
Strategies to eradicate CSCs are an urgent topic in cancer research

Cancer Stem Cell Markers

Type of cancer	CSC markers
Colon cancer	CD44+ CD133+
Breast cancer	CD44+ CD24-/low
Gastric cancer	CD44+
Pancreatic cancer	CD44+ CD24+ ESA+
Hepatic cancer	CD133+
Prostate cancer	CD44+
Metastatic melanoma	CD20+
Head and neck cancer	CD44+
Brain tumor	CD133+
Acute myeloid leukemia	CD34+ CD38-

Flow Cytometry and Cell Sorting with Anti CD44 v9 (RV3) and CD44 v10-e16 (RM1) mAb

These well characterized CD44v monoclonal antibodies are highly recommended for measuring CD44v expression by flow cytometry and for enrichment of CSC populations by cell sorting.



References

1. Nagano O., *et al., Oncogene.* 2013 Jan 21., **1**-8.
2. Tsugawa H., *et al., Cell Host Microbe.* 2012 Dec 13; **12** (6): 764-77.
3. Yae T, *et al., Nat Commun.* 2012 Jun 6; **3**: 883.
4. Ishimoto T., *et al., Cancer Cell.* 2011 Mar 8; **19** (3): 387-400.
5. Lo M, Wang YZ., *et al., J Cell Physiol.* 2008 Jun; **215** (3): 593-602.
6. Li C, Heidt DG., *et al., Cancer Res.* 2007 Feb 1; **67** (3): 1030-7.
7. Dalerba P., *et al., Proc Natl Acad Sci USA.* 2007 Jun **12**; 104 (24): 10158-63.
8. Prince ME., *et al.*, Proc *Natl Acad Sci USA.* 2007 Jan **16**; 104 (3): 973-8.
9. Anne T. Collins., *et al., Cancer Res.* 2005 Dec 1; **65** (23): 10946-51.
10. Tanabe KK., *et al., Lancet.* 1993 Mar 20; **341** (8847): 725-6.

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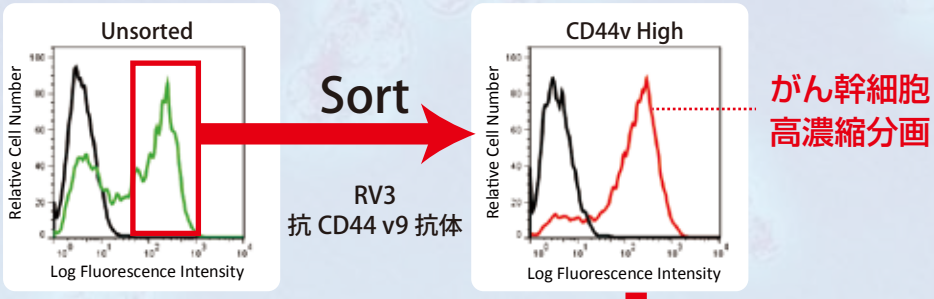
がん幹細胞が濃縮できます!

がん幹細胞濃縮抗体

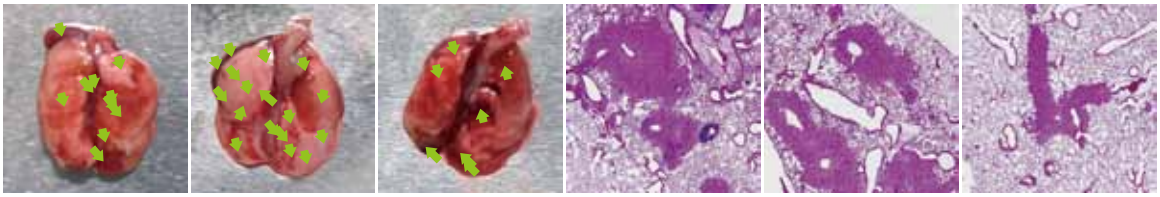
抗ヒト CD44 v9 抗体 (clone : RV3)
抗マウス CD44 v10-e16 抗体 (clone : RM1)

各種臓器の**がん幹細胞（Cancer Stem Cell: CSC）の大量濃縮**が可能となりました。がん幹細胞のマーカーとして注目されている CD44 のがん特異的なバリアントである **CD44 v に対する抗体**により、がん幹細胞の大量濃縮ができます。本抗体により、がん幹細胞に対する**治療薬開発**や**基礎研究の進展**が期待されます。

クローン RV3 による膵臓がん細胞（AsPC-1）の転移実験

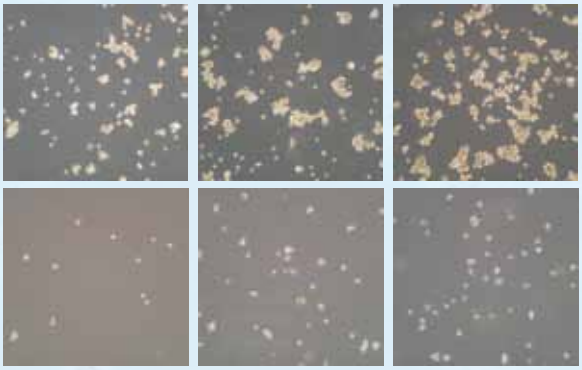


高濃縮分画による転移実験　　がん幹細胞に依存した肺転移巣の高頻度形成



がん幹細胞の持つ転移巣形成能を基にした薬剤の評価実験系の構築が可能です

クローン RV3 による前立腺がん細胞（PC-3）の Sphere 形成実験



がん幹細胞
高濃縮分画 → 低濃度播種実験
がん幹細胞に依存した腫瘍形成
（sphere）

がん幹細胞
低分率分画

薬剤の添加による Sphere の減少を測定することで薬剤の CSC への作用効果の評価が可能です。

1,250　　2,500　　5,000　　cells/well

品名	免疫動物	クローン	適用	品番	包装	希望販売価格
Anti Human CD44 v9	Rat	RV3	FCM/ IHC/ IF/WB/ IP/ ELISA	LKG-M001	100 µg	￥100,000
Anti Mouse CD44 v10-e16	Rat	RM1	FCM	LKG-M002	100 µg	￥100,000

コスモ・バイオ株式会社　　メーカー略号：CAC

詳しくは、コスモバイオの HP をご覧ください。

コスモバイオ　CD44 v　検　索

人と科学のステキな未来へ
コスモ・バイオ株式会社

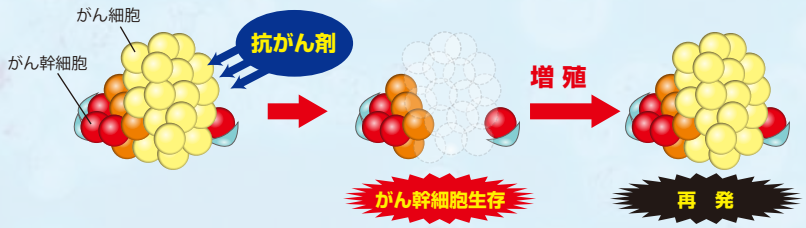
がん幹細胞が濃縮できます！

がん幹細胞濃縮抗体

抗ヒト CD44 v9 抗体 (clone : RV3)
抗マウス CD44 v10-e16 抗体 (clone : RM1)

がん幹細胞（Cancer Stem Cell）

- がん組織や細胞株中に **0.1 ～数%（微量）** 存在
- 自己複製能と無限増殖能を保有
- 抗がん剤、放射線、外部ストレス等への**耐性**
- **腫瘍形成能**を保有
- がん細胞：**転移**して転移巣形成が可能⇒**再発**の原因となっている



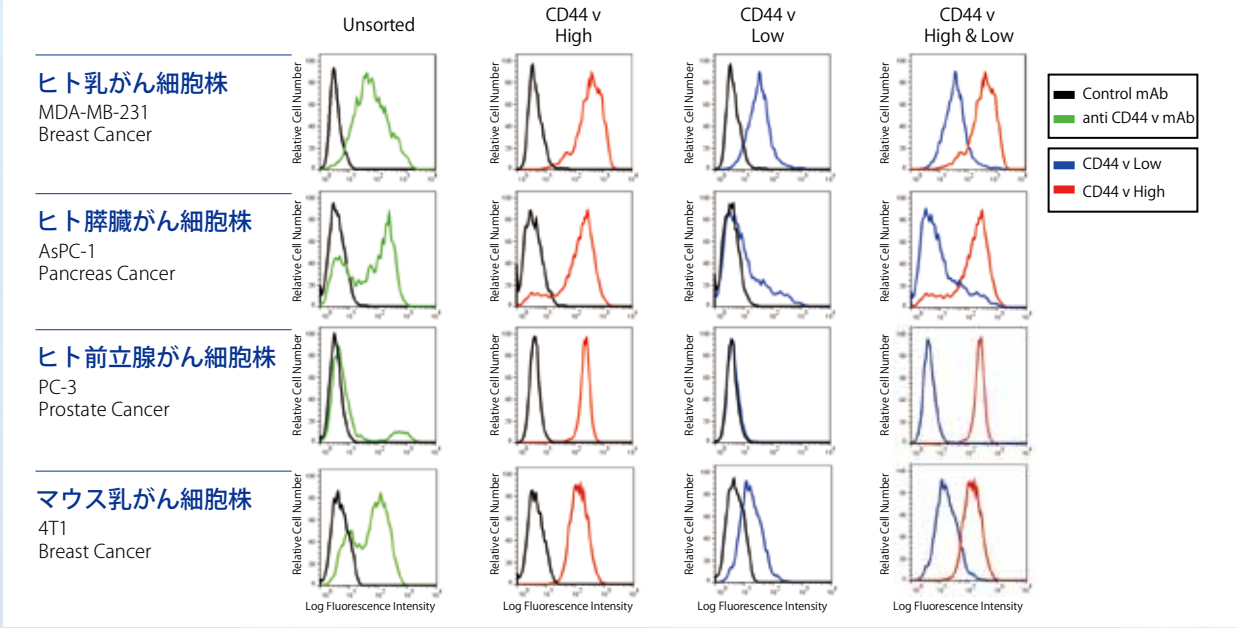
がんの死者の 90% 以上が転移・再発

がん幹細胞の治療薬の開発 ― がん治療薬開発の緊急課題

がん幹細胞 (Cancer Stem Cell) のマーカー

Type of cancer	CSC markers
大腸がん Colon cancer	**CD44+** CD133+
乳がん Breast cancer	**CD44+** CD24-/low
胃がん Gastric cancer	**CD44+**
膵臓がん Pancreatic cancer	**CD44+** CD24+ ESA+
肝がん Hepatic cancer	CD133+
前立腺がん Prostate cancer	**CD44+**
転移性悪性黒色腫 Metastatic melanoma	CD20+
頭頸部がん Head and neck cancer	**CD44+**
脳腫瘍 Brain tumor	CD133+
急性骨髄白血病 Acute myeloid leukemia	CD34+ CD38-

抗 CD44 v 抗体によるがん幹細胞 (CD44 v 高発現 CSC) の濃縮例



参考文献

1. Nagano O., *et al.*, *Oncogene.* 2013 Jan 21., 1-8.
2. Tsugawa H., *et al.*, *Cell Host Microbe.* 2012 Dec 13; **12** (6): 764-77.
3. Yae T., *et al.*, *Nat Commun.* 2012 Jun 6; **3**: 883.
4. Ishimoto T., *et al.*, *Cancer Cell.* 2011 Mar 8; **19** (3): 387-400.
5. Lo M, Wang YZ., *et al.*, *J Cell Physiol.* 2008 Jun; **215** (3): 593-602.
6. Li C, Heidt DG., *et al.*, *Cancer Res.* 2007 Feb 1; **67** (3): 1030-7.
7. Dalerba P., *et al.*, *Proc Natl Acad Sci USA.* 2007 Jun **12**; 104 (24): 10158-63.
8. Prince ME., *et al.*, Proc *Natl Acad Sci USA.* 2007 Jan **16**; 104 (3): 973-8.
9. Anne T. Collins., *et al.*, *Cancer Res.* 2005 Dec 1; **65** (23): 10946-51.
10. Tanabe KK., *et al.*, *Lancet.* 1993 Mar 20; **341** (8847): 725-6.

お願い および 注意事項

(11905)

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