

CD137

Concentrated and Prediluted Monoclonal Antibody
901-3201-090817

BIOCARE
M E D I C A L

Catalog Number:	ACI 3201 A, B	API 3201 AA
Description:	0.1, 0.5 ml, concentrated	6.0 ml, prediluted
Dilution:	1:100	Ready-to-use
Diluent:	Van Gogh Yellow	N/A

Intended Use:

For In Vitro Diagnostic Use

CD137 [BBK-2] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of 4-1BB (CD137) protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:

CD137, a member of the tumor necrosis factor receptor superfamily, represents a promising target for enhancing antitumor immune responses (1). The functions of CD137 in T lymphocytes include regulating activation, proliferation and apoptosis (2). CD137 helps regulate the activation of many immune cells, including CD4(+) T cells, CD8(+) T cells, dendritic cells, and natural killer cells. Recent studies indicate that the antitumor efficacy of therapeutic tumor-targeting antibodies can be augmented by the addition of agonistic antibodies targeting CD137. As ligation of CD137 provides a costimulatory signal in multiple immune cell subsets, CD137 antibody has potential to improve cancer treatment, and has been implicated in breast cancer, melanoma and lymphoma (3-5). Therefore, CD137 agonists represent a promising immunotherapeutic approach to treating cancers.

Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Mouse monoclonal

Species Reactivity: Human; others not tested

Clone: BBK-2

Isotype: IgG1/kappa

Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration.

Epitope/Antigen: Ectodomain of human 4-1BB recombinant protein

Cellular Localization: Cell surface

Positive Tissue Control: Small intestine with Peyer's patches, tonsil

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative

Storage and Stability:

Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations:

Peroxide Block:

Block for 5 minutes with Biocare's Peroxidized 1.

Pretreatment: Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's Background Punisher.

Primary Antibody: Incubate for 30 minutes at RT.

Probe: Incubate for 10 minutes at RT with a secondary probe.

Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.

Chromogen:

Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Biocare's Warp Red.

Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Technical Note:

This antibody has been standardized with Biocare's MACH 4 detection system. Use TBS buffer for washing steps.

Performance Characteristics:

Sensitivity and specificity on diseased tissue and tissue cross-reactivity on normal tissue is summarized in Tables 1 and 2, respectively.

Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN₃) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (6)

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Precautions Cont'd:

- Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (7)
- Microbial contamination of reagents may result in an increase in nonspecific staining.
- Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
- Do not use reagent after the expiration date printed on the vial.
- The SDS is available upon request and is located at <http://biocare.net>.

Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:

- Yonezawa A, *et al.* Boosting cancer immunotherapy with anti-CD137 antibody therapy. *Clin Cancer Res.* 2015 Jul 15;21(14):3113-20.
- Hurtado JC, *et al.* Potential role of 4-1BB in T cell activation. Comparison with the costimulatory molecule CD28. *J Immunol.* 1995 Oct 1;155(7):3360-7.
- Martinet O, *et al.* T cell activation with systemic agonistic antibody versus local 4-1BB ligand gene delivery combined with interleukin-12 eradicate liver metastases of breast cancer. *Gene Ther.* 2002 Jun;9(12):786-92.
- Li SY, Liu Y. Immunotherapy of melanoma with the immune costimulatory monoclonal antibodies targeting CD137. *Clin Pharmacol.* 2013 Sep 2;5(Suppl 1):47-53.
- Zhao S, *et al.* CD137 ligand is expressed in primary and secondary lymphoid follicles and in B-cell lymphomas: diagnostic and therapeutic implications. *Am J Surg Pathol.* 2013 Feb;37(2):250-8.
- Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
- Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

Table 1: Sensitivity and specificity was determined by testing formalin-fixed, paraffin-embedded diseased tissues.

Tissue	Positive Cases	Total Cases
Bladder Cancer	0	12
Breast Cancer	0	7
Colon Cancer	0	17
Lung Cancer	0	17
Melanoma	4	8
Ovarian Cancer	0	11
Prostate Cancer	0	15
Renal Cancer	0	9

Note: Tumor infiltrating lymphocytes are positive.

Table 2: Tissue cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	Positive Cases	Total Cases
Cerebrum	0	3
Cerebellum	0	3
Adrenal	0	3
Ovary	0	3
Pancreas	3	3
Parathyroid	0	3
Pituitary	0	3
Testis	0	3
Thyroid	0	3
Breast	0	3
Spleen	3	3
Tonsil	3	3
Thymus	3	3
Bone Marrow	0	3
Lung	0	3
Heart	0	3
Esophagus	0	1
Stomach	0	3
Small Intestine	0	3
Colon	0	3
Liver	0	3
Salivary Gland	0	3
Kidney	0	3
Prostate	0	3
Uterus	0	3
Cervix	0	3
Skeletal Muscle	0	3
Skin	2	3
Peripheral Nerve	0	3
Linging Cells	0	3