

Datasheet: MCA1724 BATCH NUMBER 166060

Description:MOUSE ANTI HUMAN CD152Specificity:CD152Other names:CTLA-4Format:PurifiedProduct Type:Monoclonal AntibodyClone:BNI3Isotype:IgG2aQuantity:0.2 mg		
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	Clone:	BNI3
Quantity: 0.2 mg	Isotype:	lgG2a
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Product Details

Applications

This product has been reported to work in the following applications. This information is derived from testing within our laboratories, peer-reviewed publications or personal communications from the originators. Please refer to references indicated for further information. For general protocol recommendations, please visit www.bio-rad-antibodies.com/protocols.

	Yes	No	Not Determined	Suggested Dilution
Flow Cytometry (1)				Neat - 1/10
Immunohistology - Frozen (2)	•			5ug/ml - 50ug/ml
Immunohistology - Paraffin		•		
ELISA	•			
Immunoprecipitation				
Western Blotting			•	
Immunofluorescence				

Where this product has not been tested for use in a particular technique this does not necessarily exclude its use in such procedures. Suggested working dilutions are given as a guide only. It is recommended that the user titrates the product for use in their own system using appropriate negative/positive controls.

- (1) Membrane permeabilization is required for this application. The use of Leucoperm (Product Code <u>BUF09</u>) is recommended for this purpose.
- (2) The epitope recognised by this antibody is reported to be sensitive to formal dehyde fixation and tissue processing. Bio-Rad recommends the use of acetone fixation for frozen sections.

Target Species	Human
Product Form	Purified IgG - liquid

Preparation	Purified by affinity chromatography on Protein A from tissue culture supernatant
Buffer Solution	Phosphate buffered saline
Preservative Stabilisers	0.09% Sodium Azide (NaN ₃)
Approx. Protein Concentrations	IgG concentration 1.0 mg/ml
Immunogen	Human CTLA-4/human IgG heavy chain fusion protein.
External Database Links	UniProt: P16410 Related reagents Entrez Gene: 1493 CTLA4 Related reagents
Synonyms	CD152
RRID	AB_2276878
Fusion Partners	Spleen cells from immunised BALB/c mice were fused with cells of the mouse P3/X63-Ag8.653 myeloma cell line.
Specificity	Mouse anti Human CD152 antibody, clone BNI3 recognizes human CD152, also known as CTLA-4 (cytotoxic T-lymphocyte-associated antigen 4), an inhibitory receptor and negative regulator of T-cell responses. CD152 is a single pass type 1 transmembrane protein belonging to the immunoglobulin superfamily containing a single <u>Ig-v-like</u> domain in the extracellular region.

CD152 along with CD28 binds to the co-stimulatory molecules CD80 and CD86 (Azuma et al. 1993).

Mouse anti human CD152 antibody, clone BNI3 is able to block ligand binding on the Raji B-cell line (Steiner et al. 2001) and blocks binding of an alternative clone, BNI8 to CTLA-4/Ig in ELISA. Mouse anti Human CD152 antibody, clone BNI3 binds to the same epitope as classified anti CTLA-4 clones 11D4 and 10A8 (Wang et al. In: Leukocyte typing VI 1997 Garland Publishing Inc. pp97-98, Bull World Health Organ. 1997).

The cytoplasmic domain of CD152 contains a critical tyrosine at residue 201 phosphorylated by Janus Kinase 2 which subsequently controls surface expression through regulation of CD152 interaction with AP-2 (Shiratori et al. 1997, Chikuma et al. 2000). CD152 is expressed primarily as an intracellular antigen with transport to the cell surface under tight regulation of several molecules including Trim, PLD and TIRC7, CD152 also demonstrates rapid internalization once expressed at the cell surface (Valk et al. 2008). CD152 plays a significant role in maintaining tolerance to self antigens and defects in CD152 presentation and expression has been implicated in a number of

autoimmune diseases (Romo-Tena et al. 2013).

This antibody can be used to develop a pharmacokinetic (PK) bridging assay to measure free fusion protein drug abatacept. It is recommended as the capture reagent, paired with human anti abatacept antibody <u>HCA335</u> as the detection antibody.

Abatacept (Orencia) is a recombinant fusion protein consisting of the extra-cellular domain of human CTLA-4, fused to the modified Fc (hinge, CH2, and CH3 domains) portion of human IgG1.

Flow Cytometry

Use 10µl of the suggested working dilution to label 1x10⁶ cells in 100µl

ELISA

Mouse anti Human CD152 antibody, clone BNI3 can be used as the capture antibody in a bridging ELISA with <u>HCA335</u> as the detection antibody.

Protocol: PK bridging ELISA

Protocol: ADA bridging ELISA

References

- 1. Linsley, P.S. *et al.* (1992) Coexpression and functional cooperation of CTLA-4 and CD28 on activated T lymphocytes. J Exp Med. 176 (6): 1595-604.
- 2. Kuiper, H.M. *et al.* (1995) Activated T cells can induce high levels of CTLA-4 expression on B cells. J Immunol. 155 (4): 1776-83.
- 3. Castan, J. *et al.* (1997) Accumulation of CTLA-4 expressing T lymphocytes in the germinal centres of human lymphoid tissues. Immunology. 90 (2): 265-71.
- 4. Steiner, K. *et al.* (1999) Enhanced expression of CTLA-4 (CD152) on CD4+ T cells in HIV infection. Clin Exp Immunol. 115 (3): 451-7.
- 5. Steiner, K. *et al.* (2001) Increased expression of CTLA-4 (CD152) by T and B lymphocytes in Wegener's granulomatosis. Clin Exp Immunol. 126: 143-50.
- 6. Pistillo, M.P. *et al.* (2003) CTLA-4 is not restricted to the lymphoid cell lineage and can function as a target molecule for apoptosis induction of leukemic cells. <u>Blood. 101: 202-9.</u>
- 7. Tan, P.H. *et al.* (2005) Creation of tolerogenic human dendritic cells via intracellular CTLA4: a novel strategy with potential in clinical immunosuppression. <u>Blood. 106:</u> 2936-43.
- 8. Lee, C.C. *et al.* (2009) The regulatory function of umbilical cord blood CD4(+) CD25(+) T cells stimulated with anti-CD3/anti-CD28 and exogenous interleukin (IL)-2 or IL-15. Pediatr Allergy Immunol. 20 (7): 624-32.
- 9. Ward, F.J. *et al.* (2013) The soluble isoform of CTLA-4 as a regulator of T-cell responses. Eur J Immunol. 43 (5): 1274-85.
- 10. Lu, C.H. *et al.* (2016) DNA Methyltransferase Inhibitor Promotes Human CD4⁺CD25^hFOXP3⁺ Regulatory T Lymphocyte Induction under Suboptimal TCR Stimulation. Front Immunol. 7: 488.

Further Reading

1. Chin, L.T. *et al.* (2008) Immune intervention with monoclonal antibodies targeting CD152 (CTLA-4) for autoimmune and malignant diseases. <u>Chang Gung Med J. 31: 1-15.</u>

Storage

This product is shipped at ambient temperature. It is recommended to aliquot and store at -20°C on receipt. When thawed, aliquot the sample as needed. Keep aliquots at 2-8°C for

short term use (up to 4 weeks) and store the remaining aliquots at -20°C.

Avoid repeated freezing and thawing as this may denature the antibody. Storage in frost-free freezers is not recommended.

Guarantee	12 months from date of despatch
Acknowledgements	Orencia is a trademark of Bristol-Myers Squibb Company
Health And Safety Information	Material Safety Datasheet documentation #10040 available at: https://www.bio-rad-antibodies.com/SDS/MCA1724 10040
Regulatory	For research purposes only

Related Products

Recommended Secondary Antibodies

Rabbit Anti Mouse IgG (STAR12...) **RPE**

Goat Anti Mouse IgG IgA IgM (STAR87...) HRP

Goat Anti Mouse IgG (STAR76...) **RPE** Goat Anti Mouse IgG (STAR70...) **FITC**

Goat Anti Mouse IgG (H/L) (STAR117...) Alk. Phos., DyLight®488, DyLight®550,

DyLight®650, DyLight®680, DyLight®800,

FITC, HRP

Rabbit Anti Mouse IgG (STAR9...) **FITC**

Goat Anti Mouse IgG (STAR77...) **HRP**

Goat Anti Mouse IgG (Fc) (STAR120...) FITC, HRP

Rabbit Anti Mouse IgG (STAR13...) **HRP**

Recommended Negative Controls

MOUSE IgG2a NEGATIVE CONTROL (MCA929)

Recommended Useful Reagents

HUMAN ANTI ABATACEPT (HCA335) HUMAN ANTI ABATACEPT (HCA336)

North & South Tel: +1 800 265 7376 America Fax: +1 919 878 3751 Worldwide

Tel: +44 (0)1865 852 700 Fax: +44 (0)1865 852 739 Europe

Tel: +49 (0) 89 8090 95 21 Fax: +49 (0) 89 8090 95 50

Email: antibody_sales_us@bio-rad.com

Email: antibody_sales_uk@bio-rad.com

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To find a batch/lot specific datasheet for this product, please use our online search tool at: bio-rad-antibodies.com/datasheets 'M422048:230901'

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