Technical Data Sheet

# Purified Hamster Anti-Mouse CD3e

## Product Information

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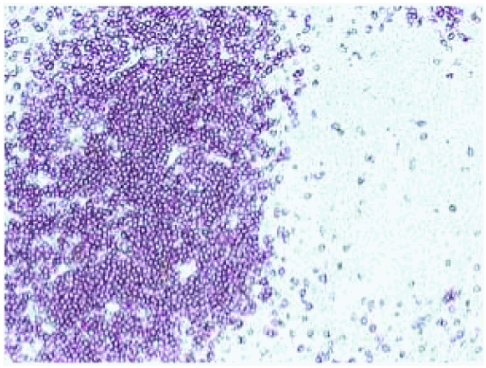
| **Material Number:** | **550275** |
| **Alternate Name:** | CD3ε chain |
| **Size:** | 1.0 ml |
| **Concentration:** | 31.25 µg/ml |
| **Clone:** | 145-2C11 |
| **Immunogen:** | H-2Kb specific cytotoxic T lymphocyte clone BM10-37 |
| **Isotype:** | Armenian Hamster IgG1, κ |
| **Reactivity:** | QC Testing: Mouse |
| **Storage Buffer:** | Aqueous buffered solution containing BSA, goat serum, and ≤0.09% sodium azide. |

## Description

The 145-2C11 antibody reacts with the 25-kDa ε chain of the T-cell receptor-associated CD3 complex, which is expressed on thymocytes, mature T lymphocytes, and NK-T cells. The cytoplasmic domain of CD3e participates in the signal transduction events which activate several cellular biochemical pathways as a result of antigen recognition. Soluble 145-2C11 antibody can activate either unprimed (naive) or primed (memory/preactivated) T cells *in vivo* or *in vitro*, in the presence of Fc receptor-bearing accessory cells. In contrast, plate-bound 145-2C11 can activate T cells in the absence of accessory cells. Soluble 145-2C11 antibody has been reported to induce re-directed lysis of Fc receptor-bearing target cells by CTL clones and can also block lysis of specific target cells by antigen-specific CTL's. Under some conditions, T-cell activation by 145-2C11 antibody has been reported to result in apoptotic cell death. The 145-2C11 antibody does not cross-react with rat leukocytes and it has been reported that pre-incubation of thymus cell suspensions at 37°C for 2-4 hours prior to staining enhances the ability of anti-CD3ε and anti-αβ TCR mAbs to detect the T-cell receptor on immature thymocytes.



***Immunohistochemical staining of T lymphocytes.*** *The frozen section of normal mouse spleen was reacted with 145-2C11 mAb. The positive staining is represented by the brown labeling of cell-surface membranes of lymphocytes in the periarteriolar sheath.*

## Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

## Application Notes

**Application**

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| Flow cytometry | Routinely Tested |
| Immunohistochemistry-frozen | Tested During Development |
| Immunohistochemistry-zinc-fixed | Tested During Development |
| (Co)-stimulation | Tested During Development |
| Immunoprecipitation | Reported |
| Western blot | Reported |
| Blocking | Reported |
| Immunohistochemistry-formalin (antigen retrieval required) | Not Recommended |

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**Recommended Assay Procedure:**

**Immunohistochemistry:** The 145-2C11 antibody is recommended to test for immunohistochemical staining of acetone-fixed frozen sections and zinc-fixed paraffin sections. Tissues tested were mouse spleen and thymus. The antibody stains the membranes of all mature T lymphocytes. The isotype control recommended for use with this antibody is purified hamster IgG, group 1, κ (Cat. No. 550344). For optimal indirect immunohistochemical staining, the 145-2C11 antibody should be titrated (1-10 to 1-50 dilution) and visualized via a three-step staining procedure in combination with biotinylated anti-hamster cocktail (Cat. No. 550335) as the secondary antibody and Streptravidin-HRP (Cat. No. 550946) together with the DAB detection system (Cat. No. 550880). **The clone 145-2C11 is not recommended for formalin-fixed paraffin embedded sections.**

For a detailed protocol, please visit the protocols section on our website at http://www.bdbiosciences.com/support/resourse.

## Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|---|---|---|---|
| 550344 | Purified Hamster IgG1, κ Isotype Control | 1.0 ml | A19-3 |
| 550335 | Biotin Mouse Anti-Hamster IgG Cocktail | 1.0 ml | G94-56 |
| 550946 | Streptavidin HRP | 50 ml | (none) |
| 550880 | DAB Substrate Kit | 500 tests | (none) |
| 552658 | 10X Zinc Fixative (Formalin Free) | 500 ml | (none) |

## Product Notices

1. Although hamster immunoglobulin isotypes have not been well defined, BD Biosciences Pharmingen has grouped Armenian and Syrian hamster IgG monoclonal antibodies according to their reactivity with a panel of mouse anti-hamster IgG mAbs. A table of the hamster IgG groups, Reactivity of Mouse Anti-Hamster Ig mAbs, may be viewed at http://www.bdbiosciences.com/documents/hamster_chart_11x17.pdf.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
5. This antibody has been developed for the immunohistochemistry application. However, a routine immunohistochemistry test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
6. An isotype control should be used at the same concentration as the antibody of interest.
7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

## References


Castro JE, Listman JA, Jacobson BA, et al. Fas modulation of apoptosis during negative selection of thymocytes. *Immunity.* 1996; 5(6):617-627. (Biology: (Co) -stimulation, Immunoprecipitation)

Isakov N, Wange RL, Burgess WH, Watts JD, Aebersold R, Samelson LE. ZAP-70 binding specificity to T cell receptor tyrosine-based activation motifs: the tandem SH2 domains of ZAP-70 bind distinct tyrosine-based activation motifs with varying affinity. *J Exp Med.* 1995; 181(1):375-380. (Biology: (Co)-stimulation, Immunoprecipitation)

Leo O, Foo M, Sachs DH, Samelson LE, Bluestone JA. Identification of a monoclonal antibody specific for a murine T3 polypeptide. *Proc Natl Acad Sci U S A.* 1987; 84(5):1374-1378. (Biology: (Co)-stimulation, Immunoprecipitation)

Nakano H, Yamazaki T, Miyatake S, Nozaki N, Kikuchi A, Saito T. Specific interaction of topoisomerase II beta and the CD3 epsilon chain of the T cell receptor complex. *J Biol Chem.* 1996; 271(11):6483-6489. (Biology: (Co)-stimulation, Immunoprecipitation)

Portoles P, Rojo J, Golby A, et al. Monoclonal antibodies to murine CD3 epsilon define distinct epitopes, one of which may interact with CD4 during T cell activation. *J Immunol.* 1989; 142(12):4169-4175. (Biology: (Co)-stimulation, Immunoprecipitation)

Radvanyi LG, Mills GB, Miller RG. Religation of the T cell receptor after primary activation of mature T cells inhibits proliferation and induces apoptotic cell death. *J Immunol.* 1993; 150(12):5704-5715. (Biology: (Co)-stimulation)

Salvadori S, Gansbacher B, Pizzimenti AM, Zier KS. Abnormal signal transduction by T cells of mice with parental tumors is not seen in mice bearing IL-2-secreting tumors. *J Immunol.* 1994; 153(11):5176-5182. (Biology: Western blot)

Shinkai Y, Alt FW. CD3 epsilon-mediated signals rescue the development of CD4+CD8+ thymocytes in RAG-2-/- mice in the absence of TCR beta chain expression. *Int Immunol.* 1994; 6(7):995-1001. (Biology: (Co)-stimulation, Immunoprecipitation)



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