

Rat Monoclonal Anti-human DPP4/CD26 Antibody E19

PRODUCT INFORMATION

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| Catalog Number: | MABS1007 |
| Hybridoma Clone: | E19 |
| Lot Number: | B-002 |
| Quantity: | 0.1 mg |
| Concentration: | 1.0 mg/mL |
| Antibody Type: | Rat IgG2a |
| Formulation: | 0.1 mg antibody in protein-free hybridoma medium, PBS, pH 7.4, and 50% glycerol. |
| Storage: | - 20° C |
| Specificity: | Human dipeptidyl-peptidase IV (DPP4, identical to adenosine deaminase complexing protein-2 and T-cell activation antigen CD26) |
| Immunogen: | DPP4 isolated from human termed placenta |
| Applications: | Immunohistochemistry, Immunocytochemistry, Immunocapture, Immunoprecipitation, Flow cytometry, ELISA, Inhibition of DPP4-gelatinase activity and cell invasion, and Western blotting. |
| Method of protein determination: | SDS-PAGE analysis showing greater than 99% protein being IgG heavy chain at 55-kDa and light chain at 25-kDa and Bradford method. |
| Method of activity determination: | Immuno-capture of recombinant antigen produced by 293-EBNA human kidney cells. |

DESCRIPTION

This antibody is produced from the E19 hybridoma cell line derived from fusion of rat myeloma Y3 cells and spleen cells of an immunized Sprague-Dawley rat (Mueller et al., 1999; Ghersi et al., 2002). DPP4 is identical to adenosine deaminase complexing protein-2 and T-cell activation antigen CD26 [Gene ID: 1803; Accession#: NP_001926]. It is a homodimeric type II membrane glycoprotein and a serine exopeptidase [EC 3.4.14.5] that cleaves X-proline dipeptides from the N-terminus of polypeptides. DPP4 was found modulating the activity of various biologically important peptides including many chemokines, neuropeptides, and peptide hormones. In addition, DPP4 acts as a receptor for adenosine deaminase (ADA), thereby mediating co-stimulatory signals in T-lymphocytes. DPP4 was also thought playing a role in physiologic glucose homeostasis that is related to type II diabetes (Marguet et al., 2000) and obesity. Recently, it was found that DPP4 and its homolog, seprase, form a novel protease complex that facilitates the local degradation and invasion of fibroblasts and endothelial cells into the extracellular matrix, suggesting a role for DPP4 in tissue repair and tumor angiogenesis (Ghersi et al., 2002; Ghersi et al., 2006).

PREPARATION

MAb E19 was produced by E19 hybridoma cells in protein-free medium using CELLLine CL 1000 Disposable Bioreactors (INTEGRA Biosciences) that includes a 10 kDa semi-permeable cellulose acetate membrane to exclude small molecules. Supernatant from the cell compartment was cleared by spinning at 10,000rpm. Approximate 5 mg/mL of antibody was obtained and diluted to 2.0 mg/mL using PBS, pH 7.4. Equal volume of glycerol was added to the antibody solution to make the final concentration of 1.0 mg/mL.

METHOD OF ACTIVITY DETERMINATION

The activity of the antibody was determined using an immunocapture assay. Briefly, anti-rat antibody was coated on a plate and 50 µg/mL of target rat monoclonal antibody was added. Recombinant human DPP4 was then added. The prolyl dipeptidase activity of the captured DPP4 was measured using Gly-Pro-p-Nitroanilide (sigma). Alternatively, antigen captured by the target antibody was detected using biotinylated anti-DPP4 antibody E26, followed by a colorimetric assay.

METHOD OF PROTEIN DETERMINATION

Protein concentration was determined using SDS-PAGE under reducing and denaturing conditions. Antibody protein was determined for its identity by SDS-PAGE analysis that shows greater than 99% of total protein being IgG heavy chain at 55-kDa and light chain at 25-kDa. The total protein in the preparation was measured with Bradford protein assay using Quick Start Bradford Dye Reagent (Bio-Rad); serially diluted BSA samples were used as standards.

STORAGE

The antibody may be stored at -20° C for one year in its original formulation. Additionally, antibody diluted with 1% BSA in PBS may be

stored at 2° to 8° C for up to 1 month without detectable loss of activity. **Avoid repeated freeze-thaw cycles of the diluted antibody.**

SPECIFICITY

MAb E19 recognizes both native and recombinant human DPP4. In Western blotting (Mueller et al., 1999; Ghersi et al., 2002) and immunoprecipitation (Ghersi et al., 2002), it does not react with human seprase, the homolog of DPP4. This antibody does not block the peptidase activity of DPP4 against Gly-Pro-pNA (Ghersi et al., 2002). It blocks the binding of DPP4 and DPP4-seprase complex to denatured type I collagen. Furthermore, this antibody inhibits gelatin degradation by the DPP4-seprase complex; it also blocks the migration and invasion of fibroblasts and endothelial cells on collagenous matrices (Ghersi et al., 2002; Ghersi et al., 2006). In addition, this antibody can block the formation of the cellular networks by endothelial cells in Matrigel (Ghersi et al., 2006).

APPLICATIONS

Immunohistochemistry – MAb E19 may be used to detect human DPP4 via immunohistochemistry techniques. Using an antibody concentration of 1 to 5 µg/mL, human DPP4 has been identified in both paraffin-embedded tissue sections and frozen sections (Ghersi et al., 2002; Ghersi et al., 2006).

Immunocytochemistry and Immunofluorescence – This antibody is effective for direct immunofluorescence staining of human DPP4 at cell surfaces (Ghersi et al., 2002; Ghersi et al., 2006).

Immuno-capture – This antibody has been employed to capture native DPP4 and recombinant DPP4 in their active forms (Ghersi et al., 2002). In the assay, the antibody is linked to a solid support to capture DPP4 in biological fluid, and the prolyl dipeptidase activity of DPP4 is measured using substrate Gly-Pro-p-Nitroanilide (sigma).

Immunoprecipitation – This antibody has been used to immuno-precipitate human DPP4 from cell or tissue lysates (Ghersi et al., 2002; Ghersi et al., 2006).

Western Blotting – This antibody can be used with the appropriate secondary reagents to detect human DPP4 in Western blotting (Ghersi et al., 2002; Ghersi et al., 2006). This antibody recognizes the 220-kDa dimers of human DPP4 under non-boiling conditions. It also reacts with DPP4 monomers under boiling conditions.

REFERENCES

- Ghersi, G., Dong, H., Goldstein, L.A., Yeh, Y., Hakkinen, L., Larjava, H.S., and Chen, W.-T. (2002). Regulation of fibroblast migration on collagenous matrix by a cell surface peptidase complex. *J. Biol. Chem.* 277, 29231-29241.
- Ghersi, G., Zhao, Q., Salamone, M., Yeh, Y., Zucker, S., and Chen, W.-T. (2006). The protease complex consisting of dipeptidyl peptidase IV and seprase plays a role in the migration and invasion of human endothelial cells in collagenous matrices. *Cancer Res* 66, 4652-4661.
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