

## ***Rat Monoclonal Anti-human DPP4/CD26 Antibody E3***

### **PRODUCT INFORMATION**

<b>Catalog Number:</b>	MABS1006
<b>Hybridoma Clone:</b>	E3
<b>Lot Number:</b>	B-001
<b>Quantity:</b>	0.1 mg
<b>Concentration:</b>	1.0 mg/mL
<b>Antibody Type:</b>	Rat IgG2a
<b>Formulation:</b>	0.1 mg antibody in protein-free hybridoma medium, PBS, pH 7.4, and 50% glycerol.
<b>Storage:</b>	- 20° C
<b>Specificity:</b>	Human dipeptidyl-peptidase IV (DPP4, identical to adenosine deaminase complexing protein-2 and T-cell activation antigen CD26)
<b>Immunogen:</b>	DPP4 isolated from human termed placenta
<b>Applications:</b>	Immunocytochemistry, Immunocapture, Flow cytometry, ELISA, Immunoprecipitation and Western blotting,
<b>Method of protein determination:</b>	SDS-PAGE analysis showing greater than 99% protein being IgG heavy chain at 55-kDa and light chain at 25-kDa and Bradford method.
<b>Method of activity determination:</b>	Immuno-capture of recombinant antigen produced by 293-EBNA human kidney cells.

### **DESCRIPTION**

This antibody is produced from the E3 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; Ghersi et al., 2006). 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 transmembrane 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 important for 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 extra-cellular matrices, suggesting a role for DPP4 in tumor invasion (Ghersi et al., 2002; Ghersi et al., 2006).

### **PREPARATION**

MAb E3 was produced by E3 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 E19, 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 E3 recognizes both native and recombinant human DPP4. In immuno-precipitation and immuno-capture assays (Gherzi 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 (Gherzi et al., 2002); neither it blocks the binding of recombinant DPP4 and DPP4-seprase complex to denatured type I collagen. Furthermore, this antibody does not inhibit the degradation of gelatin by the DPP4-seprase complex; neither it blocks the migration and invasion of fibroblasts and endothelial cells on collagenous matrices (Gherzi et al., 2002; Gherzi et al., 2006).

## APPLICATIONS

**Immunocytochemistry and Immunofluorescence** – This antibody can be used for direct immunofluorescence staining of human DPP4 on cell surfaces (Gherzi et al., 2006).

**Immuno-capture** – MAb E3 has been employed to capture native DPP4 and recombinant DPP4 in their active forms (Gherzi et al., 2002). In the assay, the antibody is linked to a solid support to capture DPP4 in cell lysates. Then 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 lysates (Gherzi et al., 2002; Gherzi et al., 2006).

**Western Blotting**–MAb E3 can be used to detect human DPP4 in Western blotting. It recognizes the dimeric and monomeric forms of human DPP4 under non-boiling and boiling conditions, respectively.

## REFERENCES

- Gherzi,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.
- Gherzi,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.
- Marguet,D., Baggio,L., Kobayashi,T., Bernard,A.M., Pierres,M., Nielsen,P.F., Ribel,U., Watanabe,T., Drucker,D.J., and Wagtmann,N. (2000). Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *PNAS* 97, 6874-6879.
- Mueller,S.C., Gherzi,G., Akiyama,S.K., Sang,Q.X., Howard,L., Pineiro-Sanchez,M., Nakahara,H., Yeh,Y., and Chen,W.-T. (1999). A novel protease-docking function of integrin at invadopodia. *J. Biol. Chem.* 274, 24947-24952.

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