

## Rat Monoclonal Anti-human DPP4/CD26 Antibody E34

### PRODUCT INFORMATION

<b>Catalog Number:</b>	MABS1005
<b>Hybridoma Clone:</b>	E34
<b>Lot Number:</b>	A-005
<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 DPP4/CD26 and seprase/DPP4 complex
<b>Immunogen:</b>	Seprase-DPP4 complex isolated from human placental cells
<b>Applications:</b>	Immunocytochemistry, Immunocapture, 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 E34 hybridoma cell line derived from fusion of rat myeloma Y3 cells and spleen cells of an immunized Sprague-Dawley rat (Nakahara et al., 1996; Nakahara et al., 1998; Nakahara et al., 1997; Mueller et al., 1999; Ghersi et al., 2002; Ghersi et al., 2006). The immunogen was purified glycoproteins from human placental cells (Nakahara et al., 1996). Further study revealed that this antibody recognizes either DPP4 or the seprase-DPP4 complex on invadopodia of LOX malignant melanoma cells that expressed low level of DPP4. Seprase, also called fibroblast activation protein alpha (FAP $\alpha$ ) [Gene ID: 2191; Accession#: NP\_004451] is a 170-kDa homodimeric integral membrane gelatinase. Both seprase and DPP4 are highly homologous and belong to the type II transmembrane serine protease family. They have a non-classical serine catalytic site and exhibit dipeptidyl dipeptidase and gelatinase activities. Seprase is selectively expressed in various cell types from cancerous tissues as well as tissues in the early stages of wound healing (Chen et al., 2003). This protein is thought to be involved in tumor invasion, angiogenesis and metastasis as well as embryo development and tissue repair. Recently, truncated and active forms of seprase have been detected in human malignant tumors (Chen et al., 2006) and plasma (Lee et al., 2006). DPP4 is the closest homolog of seprase. It is identical to adenosine deaminase complexing protein-2 and T-cell activation antigen CD26 [Gene ID: 1803; Accession#: NP\_001926]. DPP4 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 the invasion of fibroblasts, endothelial cells and epithelial tumor cells into the extracellular matrix (Ghersi et al., 2002; Ghersi et al., 2006).

### PREPARATION

Mab E34 was produced by E34 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  $\mu$ g/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 recognizes a special epitope of DPP4 that is exposed on the seprase-DPP4 complex. MAb E34 can immuno-precipitate both seprase and DPP4 in hetero-oligo dimeric forms.

## APPLICATIONS

**Immunocytochemistry and Immunofluorescence** – This antibody is effective for direct immunofluorescence staining of human DPP4 and seprase/DPP4 complex on cell surfaces (Nakahara et al., 1996).

**Immuno-capture** – This antibody has been employed to capture DPP4 and seprase/DPP4 complex in their active forms.

**Immunoprecipitation** – This antibody can immuno-precipitate seprase, DPP4 and seprase/DPP4 complex from cell or tissue lysates.

**Western Blotting** – MAb E34 can be used with the appropriate secondary reagents to detect DPP4 and seprase/DPP4 complex in Western blotting. It recognizes seprase/DPP4 complex, and dimeric forms of DPP4 under non-denaturing, non-boiling conditions. It also recognizes dissociated DPP4 monomers.

## REFERENCES

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