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Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA
Tel/Fax: (716) 829-3106 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Dipeptidyl Peptidase-4 (DPP-4; CD26) Assay Kit (Cat #: E-131)

COMPONENTS: 20x DPP-4 Buffer- 0.5 ml, store at 4ºC
10x Cell Lysis Solution- 25 ml, store at 4ºC **(contains 1% TX-100; swirl bottle briefly prior to dilution)**
100x DPP-4 Substrate- 50 µl, store at -20ºC (for 100 wells; **contains DMSO**)

PRODUCT DESCRIPTION: Dipeptidyl peptidase-4 (DPP-4; CD26) is a serine exopeptidase expressed on the surface of most cell types, capable of cleaving X-proline or X-alanine dipeptides from the N-terminus of polypeptides. The DPP-4 activity assay kit is based on proteolytic hydrolysis of the chromogenic dipeptide substrate Gly-Pro-p-nitroanilide (GP-pNA). Cleavage of pNA from the dipeptide increases absorbance at 405 nm (extinction coefficient= 9.96 $mM^{-1}cm^{-1}$), allowing for sensitive and quantitative assay of DPP-4 activity present in tissue/cell lysates and biological fluids such as serum/plasma. Both kinetic and endpoint determinations can be performed. Kit components are stable for at least 1 year if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold ddH_2O. Bring up at least ~10^5 washed cells in 100 – 200 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~10 mg tissue in 0.5 ml).

2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.

3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.5 – 1 mg/ml.

Preparation of working solution:

Each well of a 96-well plate requires 50 µl of freshly prepared working solution. For preparation of 1 ml of working solution, for example, add 50 µl 20x DPP-4 Buffer to 950 µl ddH_2O followed by addition of 10 µl 100x DPP-4 Substrate. Mix contents by vortexing and use the working solution immediately.

Enzyme assay (endpoint measurement):

1. Use a plain (uncoated) 96-well plate for the assay. Add 10 µl of each sample to the plate.

2. Calculate the amount of working solution required for each assay, and prepare the solution as described above. Swiftly add 50 µl working solution to each sample well. Mix contents by brief gentle agitation for 10 sec. IMMEDIATELY measure $O.D._{405\ nm}$ using a plate reader. Data are recorded as $O.D._{0\ min}$.

3. Cover plate and incubate at 37°C for 30 or 60 min (do not use CO_2 incubator). Measure $O.D._{405\ nm}$ after incubation. Data are recorded as $O.D._{30\ min}$ or $O.D._{60\ min}$.

4. If incubation for 30 min, DPP-4 activity in IU/L = **$(O.D._{30\ min} - O.D._{0\ min})$** x 1000 x 60 µl / (30 min x 0.3 cm x 9.96 x 10 µl) = **$(O.D._{30\ min} - O.D._{0\ min}) \times 66.93$**. If incubation for 60 min, DPP-4 activity in IU/L = **$(O.D._{60\ min} - O.D._{0\ min}) \times 33.47$**

   For tissue/cell lysates, enzyme activity should be normalized by protein concentration, and can be presented as units/µg proteins.

Additional information:

- The 100x DPP-4 Substrate solution contains DMSO. Please refer to the product page of our website or contact us for MSDS information of DMSO.