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Alanine aminopeptidase (AAP, APN, or CD13) Assay Kit (Cat #: E-145)

COMPONENTS: 20x AAP 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 AAP Substrate- 50 µl, store at -20ºC (for 100 wells; **contains DMSO**)

PRODUCT DESCRIPTION: Alanine aminopeptidase (AAP; aminopeptidase N or CD13) belongs to the group of exopeptidases that split single amino acids from the N-terminus of the peptide chain. The AAP activity assay kit is based on proteolytic hydrolysis of the chromogenic substrate L-Ala-p-nitroanilide. Cleavage of nitroanilide from the substrate increases absorbance at 405 nm (extinction coefficient= 9.96 $mM^{-1}cm^{-1}$), allowing for sensitive and quantitative assay of AAP 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 dH_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 AAP Buffer to 950 µl ddH_2O followed by addition of 10 µl 100x AAP Substrate. Mix contents by vortexing and use the working solution immediately.

Enzyme assay (endpoint measurement):

1. Place a plain (uncoated) 96-well plate on ice. 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. Blot dry bottom of plate if necessary. 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, AAP 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) = **$(O.D._{30\ min} - O.D._{0\ min})$ x 66.93**. If incubation for 60 min, AAP 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 AAP Substrate solution contains DMSO. Please refer to the product page of our website or contact us for MSDS information of DMSO.