

BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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

COMPONENTS: 20x AAP Buffer- 0.5 ml, store at 4°C
100x AAP Substrate- 50 µl, store at -20°C (for 100 wells)
10x Cell Lysis Solution- 25 ml, store at room temperature (**agitate vial to redissolve contents**)

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= $9.96 \text{ mM}^{-1}\text{cm}^{-1}$), allowing for measurement 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 dH₂O. Bring up $\sim 10^5$ washed cells in 50 – 100 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 $\sim 14,000$ rpm for 5 min. Supernatant is harvested and stored at -80°C.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 1 – 4 mg/ml. *Normalization of lysate protein concentration is recommended.*

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 ddH₂O 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 one sheet of Kimwipe positioned 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 and 60 min (do not use CO₂ incubator). Measure O.D._{405 nm} after incubation. Data are recorded as O.D._{30 min} and O.D._{60 min}.
4. If incubation for 30 min, AAP activity in IU/L = $(\text{O.D.}_{30 \text{ min}} - \text{O.D.}_{0 \text{ min}}) \times 1000 \times 60 \mu\text{l} / (30 \text{ min} \times 0.3 \text{ cm} \times 9.96 \times 10 \mu\text{l}) = (\text{O.D.}_{30 \text{ min}} - \text{O.D.}_{0 \text{ min}}) \times 66.93$. If incubation for 60 min, AAP activity in IU/L = $(\text{O.D.}_{60 \text{ min}} - \text{O.D.}_{0 \text{ min}}) \times 33.47$

For tissue/cell lysates, enzyme activity may be normalized by GAPDH enzyme activity (#E-101) or protein concentration (#A-117).

Additional information:

- The 100x AAP Substrate solution contains DMSO. Please refer to the product page of our website or contact us for SDS information.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.