BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Cathepsin B (CTSB) Assay kit (Cat #: E-146)

COMPONENTS: 10x CTSB Buffer- 0.6 ml, store at room temperature
100x CTSB Substrate- 50 µl, store at -20ºC (for 100 wells)
100x DTT: 0.1 ml, store at -20ºC
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: Cathepsin B (CTSB) is a lysosomal cysteine protease of the papain family. The CTSB activity assay kit is based on proteolytic hydrolysis of the chromogenic peptide substrate z-Arg-Arg-p-nitroanilide. Cleavage of nitroanilide from the substrate increases absorbance at 405 nm (extinction coefficient= 9.96 $mM^{-1}cm^{-1}$), allowing for measurement of CTSB activity present in tissue/cell lysates and biological fluids such as serum/plasma. Kit components are stable for several years 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 ~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 0.2 – 2 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 100 µl 10x CTSB Buffer to 900 µl ddH_2O followed by addition of 10 µl 100x DTT and 10 µl 100x CTSB Substrate. Mix contents well and use the working solution immediately.

Enzyme assay:

1. Calculate the amount of working solution required for each assay, and prepare the solution as described above.

2. Place a plain (uncoated) 96-well plate on one sheet of Kimwipe positioned on ice. Add 10 µl of each sample to the plate.

3. Add 50 µl working solution to each sample well. Mix contents by brief gentle agitation for 10 sec. Blot dry bottom of plate. IMMEDIATELY measure $O.D._{405\ nm}$ using a plate reader. Data are recorded as $O.D._{0\ min}$.

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

5. If incubation for 30 min, CTSB 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, CTSB activity in IU/L = **$(O.D._{60\ min} - O.D._{0\ min}) \times 33.47$**

   Enzyme activity can be normalized by protein concentration and presented as units/µg proteins. Alternatively, enzyme activity may be normalized by GAPDH enzyme activity (see cat#E-101).

Additional information:

- The 100x CTSB Substrate solution contains dimethyl sulfoxide (DMSO). Please refer to the product webpage or contact us for SDS information.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.