

# BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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## Cathepsin C (CTSC or DPP-1) Assay kit (Cat #: E-147)

**COMPONENTS:** 10x CTSC Buffer- 0.6 ml, store at room temp (**vortex solution prior to pipetting; turbidity is normal**)  
100x CTSC Substrate- 50 µl, store at -20°C (for 100 wells)  
100x DTT: 0.1 ml, store at -20°C  
10x Cell Lysis Solution- 25 ml, store at room temperature (**agitate vial to redissolve contents**)

**PRODUCT DESCRIPTION:** Cathepsin C (CTSC or DPP-1) is a lysosomal cysteine protease of the papain family. The CTSC activity assay kit is based on proteolytic hydrolysis of the chromogenic peptide substrate Gly-Phe-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 CTSC activity present in tissue/cell lysates. Kit components are stable for at least one 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<sub>2</sub>O. Bring up  $\sim 10^5$  washed cells in  $\sim 100 \mu\text{l}$  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 ( $\sim 10 \text{ mg}$  tissue in  $0.5 \text{ ml}$ ).
2. Centrifuge lysate in a cold microfuge at  $\sim 14,000 \text{ rpm}$  for 5 min. Supernatant is harvested and stored at  $-80^\circ\text{C}$ .
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is  $0.2 - 2 \text{ mg/ml}$ , but it should be optimized by end users.

### Preparation of working solution:

Each well of a 96-well plate requires  $50 \mu\text{l}$  of freshly prepared working solution. For preparation of  $1 \text{ ml}$  of working solution, for example, add  $100 \mu\text{l}$  10x CTSC Buffer to  $900 \mu\text{l}$  ddH<sub>2</sub>O followed by addition of  $10 \mu\text{l}$  100x DTT and  $10 \mu\text{l}$  100x CTSC Substrate. Mix contents 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  $10 \mu\text{l}$  of each sample to the plate.
3. Add  $50 \mu\text{l}$  working solution to each sample well. Mix contents by brief gentle agitation for 10 sec. Blot dry bottom of plate if necessary. Measure O.D.<sub>405 nm</sub> using a plate reader. Data are recorded as O.D.<sub>0 min</sub>.
4. Cover plate and incubate at  $37^\circ\text{C}$  for 30 min and 60 min. *Do not use CO<sub>2</sub> incubator.* Measure O.D.<sub>405 nm</sub> after incubation. Data are recorded as O.D.<sub>30 min</sub> and O.D.<sub>60 min</sub>.
5. If incubation for 30 min, CTSC 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, CTSC activity in IU/L =  $(\text{O.D.}_{60 \text{ min}} - \text{O.D.}_{0 \text{ min}}) \times 33.47$

Enzyme activity can be normalized by protein concentration, and can be presented as units/ $\mu\text{g}$  proteins. Alternatively, enzyme activity may be normalized by GAPDH enzyme activity (see cat#E-101).

### Additional information:

- The 100x CTSC Substrate solution contains 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.