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Proteasome Assay in Cell Lysates

Pamela Maher*

Cellular Neurobiology, Salk Institute for Biological Studies, La Jolla, USA

Abstract

The ubiquitin-proteasome system (UPS) mediates the majority of the proteolysis seen in the cytoplasm and nucleus of mammalian cells. As such it plays an important role in the regulation of a variety of physiological and pathophysiological processes including tumorigenesis, inflammation and cell death (Ciechanover, 2005; Kisselev and Goldberg, 2001). A number of recent studies have shown that proteasome activity is decreased in a variety of neurological disorders including Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis and stroke as well as during normal aging (Chung *et al.*, 2001; Ciechanover and Brundin, 2003; Betarbet *et al.*, 2005). This decrease in proteasome activity is thought to play a critical role in the accumulation of abnormal and oxidized proteins. Protein clearance by the UPS involves two sequential reactions. The first is the tagging of protein lysine residues with ubiquitin (Ub) and the second is the subsequent degradation of the tagged proteins by the proteasome. We herein describe an assay for the second of these two reactions (Valera *et al.*, 2013). This assay uses fluorogenic substrates for each of the three activities of the proteasome: chymotrypsin-like activity, trypsin-like activity and caspase-like activity. Cleavage of the fluorophore from the substrate by the proteasome results in fluorescence that can be detected with a fluorescent plate reader.

Materials and Reagents

1. Cells
2. HEPES (Sigma-Aldrich, catalog number: H3375)
3. MgCl₂ (Sigma-Aldrich, catalog number: M2670)
4. EDTA (Sigma-Aldrich, catalog number: E5134)
5. EGTA (Sigma-Aldrich, catalog number: E4378)
6. Sucrose (MP Biomedicals, catalog number: 821713)
7. DTT (Life Technologies, catalog number: 15508013)
8. Proteasome substrates [dissolved in DMSO (Sigma-Aldrich, catalog number: D8418) to a final concentration of 10 mM and stored frozen at -20 °C]
 - a. Suc-LLVY-AMC (chymotrypsin-like activity substrate) (Enzo Life Sciences, catalog number: P802)

*For correspondence: pmaher@salk.edu.

2. Add proteasome substrates to assay buffer (2.5 μ l/sample; 100 μ M final concentration) (make up slightly more than you need; *e.g.* if you have 12 samples, make up enough for 13).
3. Put 2 \times 200 μ l aliquots of assay buffer with substrate into 2 wells of a black walled 96 well plate for each sample.
4. Add 50 μ l cell lysate to each well (include 2 wells with lysis buffer with no cells as assay blanks).
5. Incubate 60 min at 37 $^{\circ}$ C in order to allow the cleavage of the fluorophore from the proteasome substrate.
6. Read $A_{360\text{ex}}/A_{460\text{em}}$ on fluorescent plate reader.
7. Normalize to protein determined with the Coomassie Protein Assay following the manufacturer's instructions for a microplate reader and using 5 μ l of lysate.

Recipes

1. Proteasome Lysis/Assay Buffer
50 mM HEPES (pH 7.8)
10 mM NaCl
1.5 mM MgCl_2
1 mM EDTA
1 mM EGTA
250 mM sucrose
Sterile filter and stored at 4 $^{\circ}$ C
For lysis buffer: add DTT to 5 mM final concentration (use 1 M stock)
For assay buffer: add DTT to 5 mM final concentration and ATP to 2 mM final concentration

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