Lot # XXXXX

BostonBiochem

# MATERIAL DATA SHEET

## 20S Proteasome Assay Kit, *SDS-Activation Format*
## Cat. # K-900

This kit contains buffers and reagents for the quantitative analysis of 20S proteasome activity in cuvettes or a 96-well microtiter plate formats. The 20S activity is measured by monitoring the release of free AMC from the fluorogenic peptide Suc-Leu-Leu-Val-Tyr-AMC **(S-280)**. The rate of AMC release may be measured either by absorbance or fluorescence over time.

NOTE: Please read instructions completely prior to performing the assay. Kit contains reagents sufficient for 96 x 200 µl reactions.

**MATERIALS NOT SUPPLIED BUT NEEDED**:

- Micro-centrifuge
- fluorescence spectrophotometer
- quartz cuvettes or flat-bottom opaque 96-well plate
- free AMC for standard curve generation and sample quantitation

| Product Information | |
|---|---|
| **Quantity/Stock:** | 1. **Enzyme Solution**, 20S Proteasome, 25 µg<br>X mg/ml (X µM). Concentration varies with Lot #.<br>2. **Reaction Buffer**, 2 x 1.5 mL<br>3. **Activation Solution**, 250 µL<br>4. **Substrate Suc-LLVY-AMC Solution**, 50 µL<br>X mM in DMSO. Concentration varies with Lot#. |
| **Purity:** | > 95% by SDS-PAGE |
| **Storage:** | Enzyme Solution should be stored at -80°C. Avoid multiple freeze/thaw cycles.<br>All other components can be stored at -20°C. |

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## Background

The Ubiquitin Proteasome Pathway (UPP) is the cell's principle mechanism for protein catabolism. The UPP has been shown to have significant involvement in the regulation of critical cellular processes such as transcription, oncogenesis, cell cycle progression, development, growth, selective elimination of abnormal proteins, and antigen processing (1-3). The proteasome is a large, multimeric protease that catalyzes the final step of the UPP intracellular protein degradation. The proteasome exists in multiple forms within the eukaryotic cell, and contained in all isoforms is the catalytic core known as the 20S proteasome. The 20S proteasome (700 kDa) is arranged as four axially stacked heptameric rings with two β-subunit rings sandwiched between two α-subunit rings. The multicatalytic centers are located within the internal cavity of the β-subunits. The 20S proteasome is characterized by three distinct proteolytic activities against short synthetic peptides: chymotryptic-like (Tyr or Phe at P1), tryptic-like (Arg or Lys at P1) and peptidylglutamyl peptide- hydrolyzing (Glu at P1) (4). These activities are believed to be catalyzed by the nucleophilic N-terminal threonines of the β-subunits (5, 6). The 20S proteasome *in vitro* cannot efficiently degrade peptides or proteins unless they are highly denatured and the 20S has been activated by the addition of low concentrations of sodium dodecyl sulfate (SDS) or PA28 (or 11 S activator) (7-12). The exact mechanism for this apparent SDS activation is not well-understood, except to postulate that SDS induces conformational changes under limited denaturation thereby allowing access to the central cavity where the active sites residues reside.

The most common assessment of 20S activity *in vitro* is done by measuring the hydrolysis of the fluorogenic peptidyl substrate Suc-Leu-Leu-Val-Tyr-AMC **(Cat# S-280)** by the SDS-activated proteasome. This substrate is cleaved by the chymotryptic-like activity of the proteasome releasing free AMC (7-amino-4-methylcoumarin) which can be efficiently detected using a fluorimeter ($Ex_{380nm}$; $Em_{460\ nm}$). Purified 20S proteasome chymotryptic-like activity is dependent on SDS concentration up to 0.030% SDS where the activity is maximal (**Fig.1**). SDS concentrations beyond 0.030% result in inactivation due to excessive and irreversible denaturation of the 20S. When this substrate is titrated against a fixed concentration of proteasome pre-activated with SDS, Suc-LLVY-AMC exhibits a $K_M$ value of 9 μM (**Fig. 2**). At saturating substrate concentrations (≥ 50 μM), the turn-over rate ($V_{max}$) is approximately 12 $s^{-1}$.

## Assay Considerations

For quantitative measurements of the proteasome, the fluorimeter may be calibrated by generating a standard curve using AMC. The standard curve should have a concentration range of 0–100 pmol AMC. This calibration allows for the calculation of exact specific activity of the 20S on each individual fluorimeter. **NOTE:** *This kit does not supply AMC for calibration.*

For optimum activity, it is recommended that the assay be performed at 37°C. However, if the measurement instrument has no temperature control, the assay may be performed at room temperature. The activity signal at 25°C will decrease approximately 3-4 fold relative to signal measured at 37°C. It is important to prepare all solutions by thawing them briefly in a warm water bath. A quick centrifugation is recommended to limit loss of materials on tube sides and caps. Vortex and/or sonicate the SDS solution briefly to ensure that it is in solution prior to use. Once thawed, the 20S enzyme solution should be placed immediately on ice. The 20S proteasome solution as supplied is stable on ice for at least 24 hours. Once activated with SDS, the activity is stable at 37°C for at least 1 hour. SDS, substrate and reaction buffer solutions can be stored at room temperature during the course of their use. Depending on what purpose this kit is being used for, the addition order of substrate, SDS, and 20S may vary. Components should be diluted into reaction buffer and mixed well prior to the addition of other kit reagents.

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Many types of agents may be tested in the assay. Although the concentration of SDS for maximal activation (0.03%) is below the critical micellar concentration (CMC), SDS still may interact with some agents (i.e. unstable proteins and highly positively charged agents). In such cases, an alternate method of activating the 20S proteasome is activator PA28 **(Cat # E-380)**. Due to the inherent decreased sensitivity of fluorimetric plate readers compared to cuvette-based fluorimeters, higher enzyme concentrations or longer incubations with substrate may be needed to produce a strong signal when using the kit in microtiter plate format.

## Literature

**References:**


1. Coux O., *et al.* (1996) Annu. Rev. Biochem. **65**:801-847
2. DeMartino G.N. and Slaughter C.A. (1999) J. Biol. Chem. **274**:22123-22126
3. Voges D., et al. (1999) Annu. Rev. Biochem. **68**:1015-1068
4. Stein RL.., *et al.* (1996) Biochem. **35**:3899-38908
5. Groll M., *et al.* (1997) Nature **386**:463-471
6. Seemuller E., *et al*. (1996) Nature **382**:468-471
7. Ambes J. and Castano J.G. (1990) J. Biol. Chem. **265**:13969-13973
8. Dahlman B. et al. (199#) Enz. Prot. 47:274-286
9. Hoffman L., *et al.* (1992) J. Biol. Chem. **267**:22362-22368
10. Ma C.P., *et al.* (1992) J. Biol. Chem. **267**:10515-10523
11. Shibatani T. and Ward W.F. (1995) Arch. Biochem. Biophys. **321**:160-166
12. Yamada S. *et al.*, (1995) J. Biochem. **117**:1162-1169


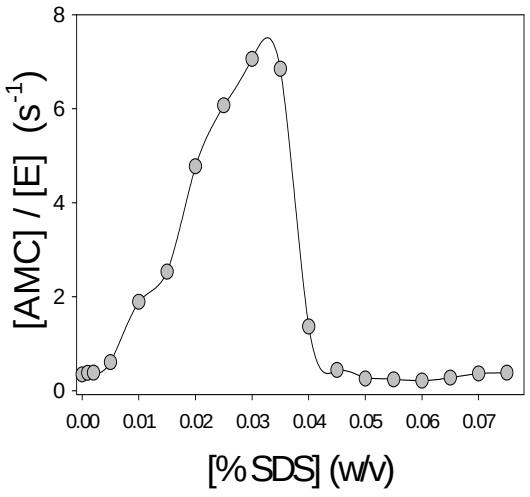


**Figure 1. SDS-activation of purified 20S Proteasome.** Proteasome (1.2nM) was preincubated with with varying concentrations of SDS at 37°C. Substrate was added and hydrolysis (rate of AMC release) was monitored at $\lambda_{Em}$380 and $\lambda_{Ex}$440nm respectively.

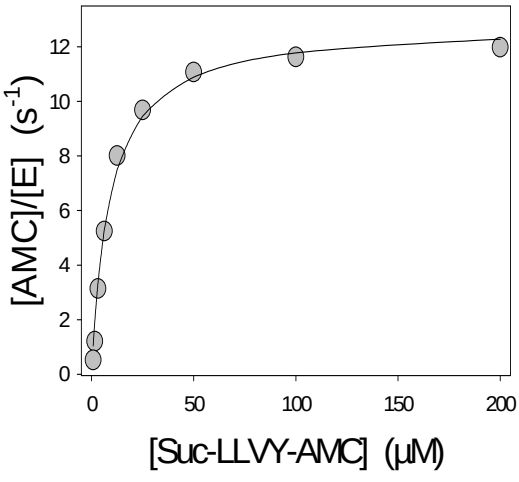


**Figure 2. Michealis-Menton parameters of Suc-LLVY-AMC with SDS-activated 20S Proteasome.** Proteasome was activated and assayed with varying concentrations of substrate (0.05-200µM) at 37°C. The rate of AMC release was monitored at $\lambda_{Em}$380 and $\lambda_{Ex}$440nm respectively.

***For Laboratory Use Only, Not For Use in Humans***


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