

# 20S Proteasome Assay Kit

*(SDS Activation Format)*



# 20S Proteasome Assay Kit

## *(SDS Activation Format)*

Catalog Number K-900

This kit contains buffers and reagents for the analysis of 20S proteasome activity in a microtiter plate format. The 20S activity is measured by monitoring the release of free AMC from the fluorogenic peptide Suc-LLVY-AMC, [\(R&D Systems®, Catalog # S-280\)](#).

This package insert must be read in its entirety before using this product.  
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION	PAGE
INTRODUCTION .....	1
TECHNICAL HINTS .....	2
PRECAUTIONS .....	2
MATERIALS PROVIDED & STORAGE CONDITIONS .....	3
OTHER MATERIALS REQUIRED .....	3
ASSAY PROTOCOL .....	4
SAMPLE DATA .....	5
REFERENCES .....	6

Manufactured and Distributed by:

**USA R&D Systems, Inc.**  
614 McKinley Place NE, Minneapolis, MN 55413  
TEL: 800 343 7475 612 379 2956  
FAX: 612 656 4400  
E-MAIL: info@bio-techne.com

Distributed by:

**Europe | Middle East | Africa Bio-Techne Ltd.**  
19 Barton Lane, Abingdon Science Park  
Abingdon OX14 3NB, UK  
TEL: +44 (0)1235 529449  
FAX: +44 (0)1235 533420  
E-MAIL: info.emea@bio-techne.com

**China Bio-Techne China Co., Ltd.**  
Unit 1901, Tower 3, Raffles City Changning Office,  
1193 Changning Road, Shanghai PRC 200051  
TEL: +86 (21) 52380373 (400) 821-3475  
FAX: +86 (21) 52371001  
E-MAIL: info.cn@bio-techne.com

## INTRODUCTION

The Ubiquitin Proteasome System (UPS) is the cell's principal mechanism for protein catabolism. The UPS is involved in the regulation of critical cellular processes such as transcription, oncogenesis, cell cycle progression, elimination of abnormal proteins, and antigen processing (1-3). The proteasome is a large, multimeric protease that catalyzes the final step of UPS-mediated 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 700 kDa 20S Proteasome is arranged as four axially stacked heptameric rings with two  $\beta$ -subunit rings sandwiched between two  $\alpha$ -subunit rings. The catalytic centers are located within the internal cavity of the  $\beta$ -subunits. The 20S Proteasome is characterized by three distinct proteolytic activities including chymotrypsin-like, trypsin-like and caspase-like (4). *In vitro*, the 20S Proteasome cannot efficiently degrade peptide substrates unless it's been activated by the addition of low concentrations of sodium dodecyl sulfate (SDS) or PA28 activators (7-12). The exact mechanism for SDS activation is not well-understood but may involve conformational changes under limited denaturation allowing access to the central cavity where the protease sites reside.

The most common assessment of 20S activity *in vitro* is done by measuring the hydrolysis of the fluorogenic peptide substrate Suc-LLVY-AMC ([R&D Systems®, Catalog # S-280](#)) by the SDS-activated proteasome. This substrate is cleaved by the chymotrypsin-like activity of the proteasome, releasing free AMC (7-amino-4-methylcoumarin dye) which can be detected using a fluorimeter with excitation and emission wavelengths of 345 nm and 445 nm, respectively. Purified 20S proteasome chymotrypsin-like activity is dependent on SDS concentration up to 0.030% SDS where the activity is maximal (Figure 1). SDS concentrations above 0.03% typically result in irreversible inactivation of 20S activity.

## TECHNICAL HINTS

- For quantitative measurements of proteasome activity using AMC-based substrates, an AMC standard curve may be useful. Typically, the standard curve should have a concentration range of 0-1  $\mu$ M AMC in the same volume and buffer conditions used for the 20S assays.
- The optimal reaction temperature for 20S proteasome activity is 37 °C, but the assay can be performed at room temperature. 20S activity at 25 °C will decrease approximately 3-4 fold relative to 37 °C assay conditions.
- 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.
- Prior to use, warm and briefly vortex the SDS solution to ensure that it is in solution.
- 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.

Measurement	Order of Addition into Cuvette or Plate
20S Inhibition	Buffer, SDS, 20S, Substrate, Inhibitor
20S Inhibition with pre-incubation	Buffer, SDS, 20S, Inhibitor, Substrate
20S Latent Activity (non-activated)	Buffer, 20S, Substrate
20S Activation by test article	Buffer, 20S, Substrate, Activator
20S Activation by test article with pre-incubation	Buffer, 20S, Activator, Substrate

- Many types of agents may be tested in the assay. Although the concentration of SDS for maximal 20S proteasome activation (0.03%) is low, SDS may interact with some agents (e.g. unstable proteins and highly positively charged agents) and cause interference in the assay. In such cases, an alternate method of activating the 20S Proteasome may be needed. One such option is the use of PA28 activator proteins (R&D Systems®, Catalog# [E-380](#) or [E-381](#)).
- Depending on the sensitivity of the plate reader used, or other experimental parameters, a higher 20S enzyme concentration, or longer incubation times may be needed to produce a robust fluorescent signal.

## PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Kit contains buffers and reagents for the analysis of 20S proteasome activity in a microtiter plate format.

COMPONENT	VOLUME	DESCRIPTION	STORAGE OF UNOPENED COMPONENTS	STORAGE OF THAWED/ ACTIVATED COMPONENTS
20S Proteasome	18 µL	1.4 mg/mL (2 µM) in 50 mM HEPES, pH 7.6, 100 mM NaCl, 1 mM DTT"	Store at ≤ -80 °C. Avoid multiple freeze-thaw cycles.	Place on ice immediately after thawing and for up to 24 hours. After SDS activation, working stocks of 20S Proteasome remain active for 1 hour or more.
20X Reaction Buffer	2 x 1.5 mL	500 mM HEPES, 10 mM EDTA, pH 7.6	Store at ≤ -20 °C.	Reaction Buffer and SDS Solution may be stored at room temperature. Substrate Solution is stable at room temperature for at least 48 hours but should be routinely stored in aliquots at ≤ -20 °C or ≤ -80 °C.
100X Activation Solution	250 µL	3% SDS (w/v) in dH <sub>2</sub> O		
1000X Substrate Solution	50 µL	10 mM Suc-LLVY-AMC in DMSO		

## OTHER MATERIALS REQUIRED

- Micro-centrifuge
- Fluorescence plate reader capable of using excitation/emission wavelengths of 345/445 nm, respectively
- 96-well, black opaque microtiter plate
- Optional: AMC solution for standard curve and sample quantitation

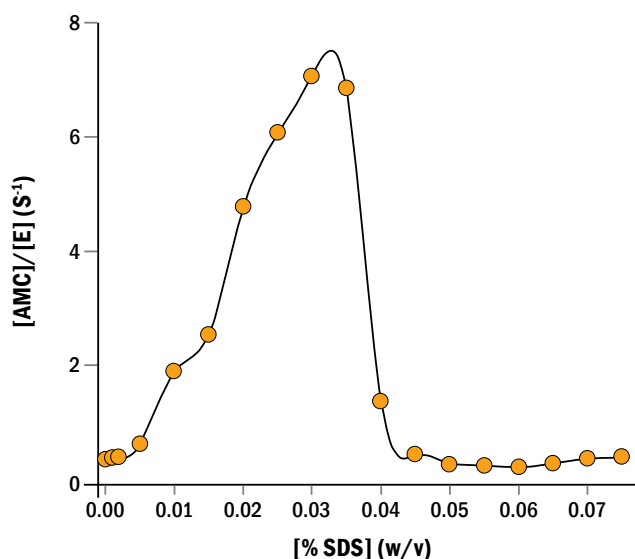
## ASSAY PROTOCOL

**Kit contains enough reagents for 1 x 96-well microtiter plate when using 200  $\mu$ L reaction volume. Reaction volumes may be scaled up or down to meet specific assay criteria.**

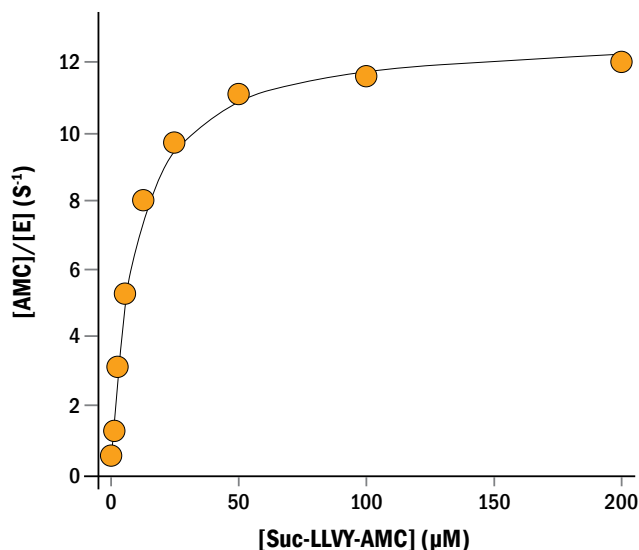
1. Thaw all solutions briefly in a warm water bath. Briefly centrifuge 20S Proteasome, flick gently to make sure it's well mixed, then place immediately on ice. All other reagents may be kept at room temperature during the assay set up.
2. Using a 15 or 50 mL conical tube, prepare the amount of 1X Reaction Buffer needed for the number of reactions planned. Each well will require a total of 200  $\mu$ L.
3. Add SDS to the Reaction Buffer such that final concentration will be 0.03% (1:100 dilution) in the assay.
4. Add Proteasome Enzyme to the Reaction Buffer/SDS mix (0.2  $\mu$ g per well) and incubate for 5-10 minutes.
5. Aliquot 190  $\mu$ L activated enzyme mixture into each well on the plate and bring plate to desired reaction temperature.
6. Make a 20X (200  $\mu$ M) Substrate Working Solution by diluting Stock Substrate 1:50 in 1X Reaction Buffer. Each well will require 10  $\mu$ L.
7. Initiate the reaction by adding 10  $\mu$ L of the 20X Substrate Working Solution to each well.
8. Monitor fluorescence signal over time using excitation and emission wavelengths of 345 nm and 445 nm, respectively.



## SAMPLE DATA



**Figure 1:** SDS-activation of purified 20S Proteasome. Proteasome (1.2 nM) was pre-incubated with reaction buffer (25 mM Hepes pH 7.6, 0.5 mM EDTA) with varying concentrations of SDS at 37 °C. 20 μM of Suc-LLYY-AMC, ([R&D Systems®, Catalog # S-280](#)) was added and hydrolysis (rate of AMC release) was monitored using excitation and emission wavelengths of 345 nm and 445 nm, respectively.



**Figure 2:** Michealis-Menton parameters of SDS-activated 20S Proteasome using Suc-LLVY-AMC ([R&D Systems®, Catalog # S-280](#)). Proteasome (1.2 nM) was activated with reaction buffer (25 mM Hepes pH 7.6, 0.5 mM EDTA, 0.03% SDS) and assayed with varying concentrations of substrate (0.05-200 μM) at 37 °C. The rate of AMC release was monitored using excitation and emission wavelengths of 345 nm and 445 nm, respectively.

## REFERENCES

1. Coux, O. *et al.* (1996) *Annu. Rev. Biochem.* **65**: 801.
2. DeMartino G.N. and Slaughter C.A. (1999) *J. Biol. Chem.* **274**: 22123.
3. Voges, D. *et al.* (1999) *Annu. Rev. Biochem.* **68**:1015-1068.
4. Stein, R.L. *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.



*All trademarks and registered trademarks are the property of their respective owners.*