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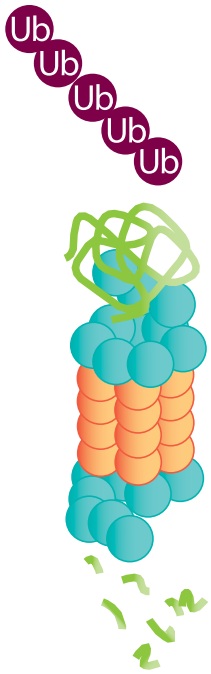
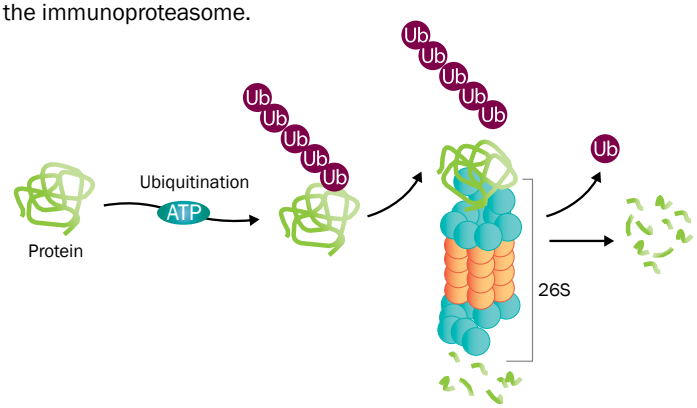
# Tools for Proteasome Research



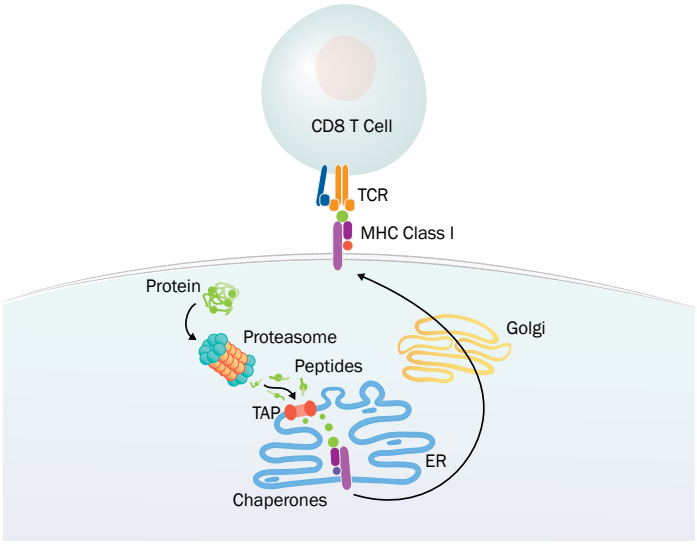
# Products for Proteasome Research

Proteasomes are intracellular, multi-subunit protein complexes found in all eukaryotes and in some bacteria. Structurally, the “20S core” of proteasomes consists of four stacked rings (28 subunits total) that form a central pore. The function of proteasomes is to degrade unneeded or damaged proteins; it does this using a series of proteases contained inside the central pore. Proteins are often tagged for degradation with a small protein called Ubiquitin. The Ubiquitin tag interacts with a series of gating subunits (“19S”) that are located at either end of the Proteasome core. This interaction results in the tagged protein being unfolded (an ATP-dependent process) and fed into the core where it is degraded into polypeptides. In higher organisms there are at least two basic types of proteasomes, The constitutive proteasome and the immunoproteasome.




**The Constitutive Proteasome.** The constitutive proteasome is widely distributed among cell types and used for household degradation of proteins.

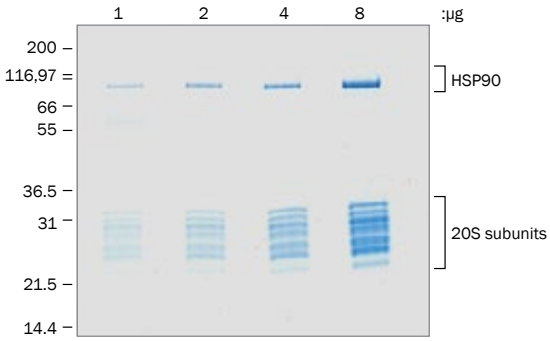
Proteasome activity is essential for cellular protein homeostasis, and plays important roles in cell-cycle control, inflammatory responses, apoptosis, heat-shock responses, and adaptive immunity. Furthermore, abnormal functioning of the Ubiquitin-Proteasome-System (UPS) contributes to a number of human pathologies including Parkinson’s Disease, various cancers, Alzheimer’s Disease, and ALS. Finally, inhibiting proteasome activity with small molecules such as Bortezomib and Carfilzomib has proven efficacious in the treatment of multiple myeloma.



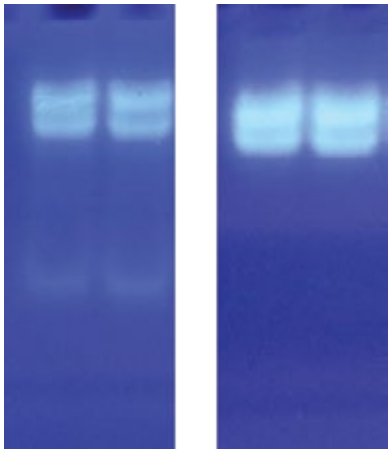

**The Immunoproteasome.** The immunoproteasome is found in antigen-presenting cells and cells that have been stimulated with interferon gamma. Compared to the constitutive proteasome, the immunoproteasome processes proteins into polypeptides that are more optimal ligands for presentation in MHC class I proteins.

# Unsurpassed Purity and Functional Characterization

Every lot of proteasome produced at Boston Biochem is tested for activity and analyzed for purity. Our extensive quality control testing ensures performance and reproducibility for your experiments.




**Proteasome Purity Highlighted with SDS-PAGE.** From 1–8 µg of human i20S proteasome (Catalog # E-370) was visualized by reducing SDS-PAGE and Colloidal Coomassie Blue staining. The 20S subunits are seen in the 25-35 kDa range, while HSP90, a tightly associated chaperone present in immunoproteasome obtained from PBMCs is seen at 116–97 kDa.



**Proteasome Activity Highlighted Using a Fluorogenic Substrate.** Human 26S proteasome (Catalog # E-365) was separated using a 5% native, non-reducing PAGE gel then analyzed for activity by soaking in LLVY-AMC substrate. **Left panel:** LLVY-AMC assay done the presence of 0.035% SDS. **Right panel:** LLVY-AMC assay done without 0.035% SDS. Digitometry values reveal that the purified proteasome consists of double-capped + single-capped species of 89%; uncapped (20S) proteasome was present at just 11%.

## Purified Proteasomes from Boston Biochem

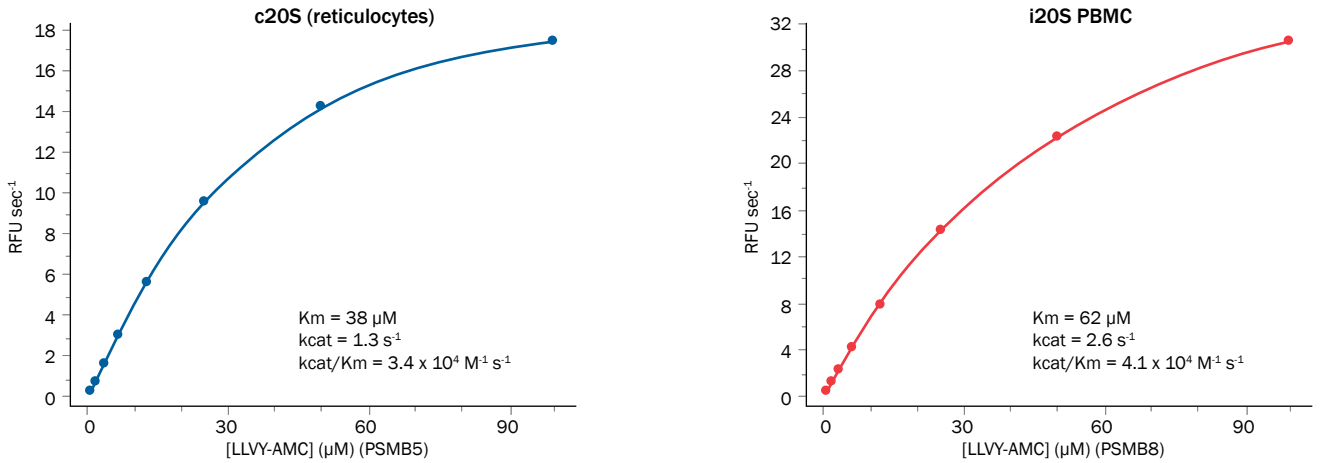
| Molecule | Species | Catalog # |
|---|---|---|
| c20S Proteasome | Rabbit | E-350 |
| | Rat | E-352 |
| | Mouse | E-355 |
| | Canine | E-358 |
| | Human | E-360 |
| | Cynomolgus | E-379 New! |

| Molecule | Species | Catalog # |
|---|---|---|
| i20S Proteasome | Human (PBMC source) | E-370 |
| | Human (Ramos source) | E-371 New! |
| | Rat | E-375 |
| | Mouse | E-376 |
| | Canine | E-377 |
| | Cynomolgus | E-378 New! |

| Molecule | Species | Catalog # |
|---|---|---|
| 26S Proteasome | Human | E-365 |
| 19S Proteasome | Human | E-366 |
| 19S Proteasome (UCHL5 C88A) | Human | E-367 |

## Fluorogenic Proteasome Substrates for Activity Testing

Peptide-AMC conjugates are excellent substrates for monitoring proteasome activity, and may be used in either end-point or continuous monitoring (kinetic) formats. Depending on the peptide sequence, some substrates may be used with either constitutive or immunoproteasomes, while others show a marked difference in hydrolysis rates between the two proteasome types.




**Proteasome Activity Measured using a Fluorogenic Subtrate.** Human 20S constitutive proteasome (left panel) and human 20S immunoproteasome (right panel) activity was determined using the LLVY-AMC Fluorogenic Substrate (Catalog # S-280). This substrate is efficiently hydrolyzed by both proteasome types.

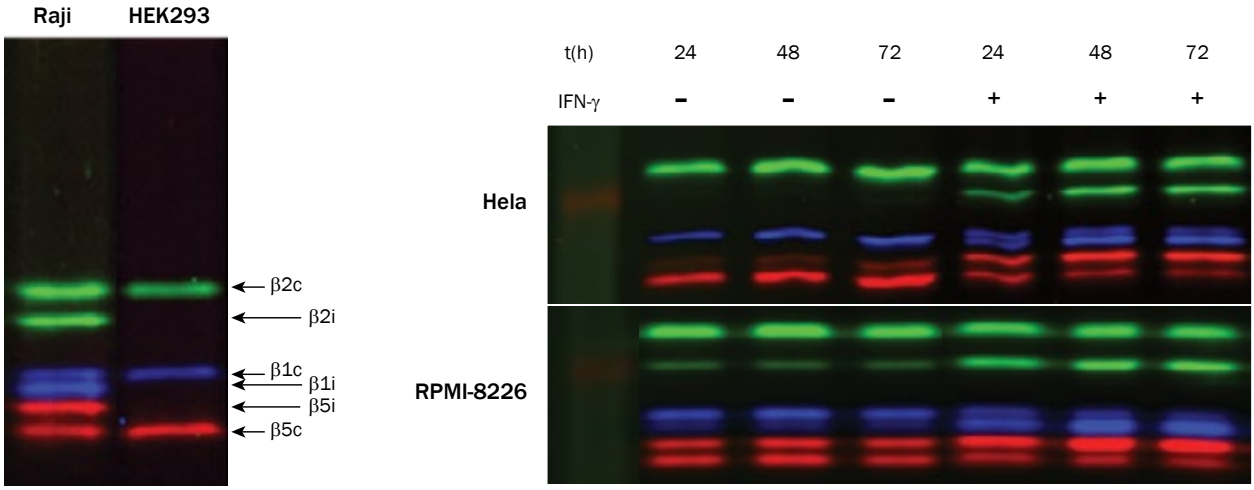
## Fluorogenic Proteasome Substrates for Activity Testing

| Substrate | Catalog # |
|---|---|
| Z-Leu-Leu-Leu-AMC (Z-LLL-AMC) | S-220 |
| Z-Leu-Leu-Glu-AMC | S-230 |
| Suc-Leu-Tyr-AMC (Suc-LY-AMC) | S-260 |
| Suc-Leu-Leu-Val-Tyr-AMC (Suc-LLVY-AMC) | S-280 |
| Ac-Arg-Leu-Arg-AMC (Ac-RLR-AMC) | S-290 |
| Boc-Leu-Arg-Arg-AMC (Boc-LRR-AMC) | S-300 |
| Ac-Pro-Ala-Leu-AMC (Ac-PAL-AMC) | S-310 |
| Ac-Ala-Asn-Trp-AMC (Ac-ANW-AMC) | S-320 |
| Ac-Trp-Leu-Ala-AMC (Ac-WLA-AMC) | S-330 |

# Coming soon:
## β-subunit specific probes

In collaboration with Hermen Overkleeft and colleagues, Boston Biochem will soon offer a panel of tagged and fluorescent probes for labeling active β-subunits of the proteasome. These compounds are useful in many *in vitro* applications, including:

- Determining levels of constitutive vs immunoproteasome activity
- Following induction of immunoproteasome activity after interferon treatment
- Monitoring inhibition of β-subunit after treating cells with proteasome inhibitors




**Labeling of Active β Subunits.** Staining profile for Raji and HEK293 cells (left panel). The three color probes indicate the presence of consitutive β subunits 2c, 1c, and 5c (green, blue, and red, respectively) in cell lysates from HEK cells, indicating that these cells only express constitutive proteasomes. The Raji cell lysates show the presence of three additional bands that correlate to the three β subunits of the immunoproteasome as well. Right Panel: IFN-γ treatment induces immunoproteasome activity. RPMI cells express both constitutive and immunoproteasome activities, with the latter being upregulated following 24 hours of treatment with IFN-γ. HeLa cells lack appreciable immunoproteasome activity in the absence of IFN-γ, but the β1i, β2i, and β5i subunits are clearly upregulated. (Figures adapted from de Bruin G. *et al.* (2015) Angew. Chem. Int. Ed. **54**:1.

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