20S Proteasome (Human)

Cat. # PS020

Background:

The proteasome is a large, multi-catalytic protease complex within the eukaryotic cell. The 26S proteasome, a 60-subunit particle is composed of a 20S core and one or two 19S regulatory particles. The catalytic core known as the 20S proteasome (700 kDa) is composed of two sets of seven β-subunits and two sets of seven α-subunits. The β1, β2 and β5 subunits possess caspase-like, trypsin-like and chymotrypsin-like peptidolytic activities respectively. In eukaryotic cells, the 20S proteasome is assembled with the aid of at least five assembly chaperones. The degradation of cellular proteins by the proteasome is initiated by attachment of an ubiquitin chain to the polypeptide. The ubiquitin tag is recognized by and binds to the 'lid', a 19S particle, followed by disassembly of ubiquitin chains, protein unfolding and subsequent translocation into the 20S proteasome.

This purified 20S proteasome preparation can be used *in vitro* for the degradation of peptide substrates and is suitable to screen for novel proteasome inhibitors. To measure a chymotrypsin activity of 20S proteasome, a specific substrate Suc-LLVY-AMC (cat.no.PS500) is available. Upon cleavage by the active enzyme, it generates a highly fluorescent product with an emission wavelength at 460 nm.

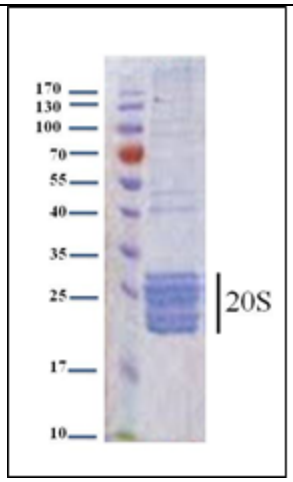
Product Information

Quantity: 50 μg, 1 mg/ml

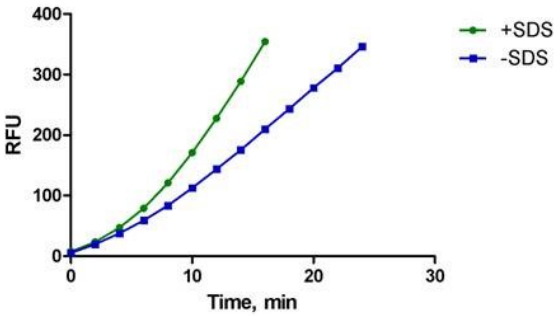
Buffer: 20 mM Tris-HCl pH 7.2, 10% Glycerol, 150 mM KCl, 1 mM β–mercaptoethanol.

Source: Human red blood cells

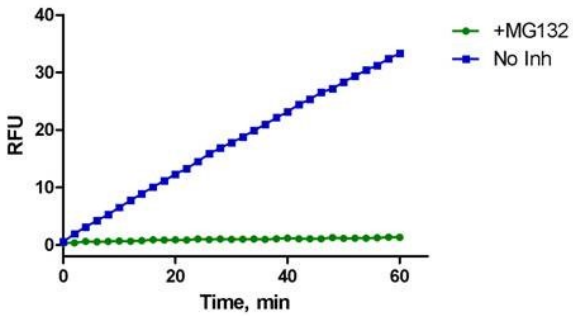
Storage: -80° C. Avoid repeated freeze/thaw cycles



20S proteasome was purified from human blood cells by DEAE chromatography follow by the ammonium sulfate precipitation (60%), Mono Q and Resource Q chromatography. Purified complex was resolved on 10% SDS polyacrylamide gel and stained with Coomassie blue.



The chymotrypsin-like activity of the 20S proteasome (45μg/ml) was measured using 100μM Suc-LLVY-AMC in 20 mM HEPEs, pH 7.5, 0.5 mM EDTA, 0.05% Triton X, plus or minus 0.035% SDS



The chymotrypsin-like activity of the 20S proteasome (18 μg/ml) was measured using 100μM Suc-LLVY-AMC in 20 mM HEPEs, pH 7.5, 0.5 mM EDTA, 0.05% Triton X, plus or minus 0.5 mM MG132 (Cat. # SI9710)

References

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2. Golgberg AL (2012) JCB Development of proteasome inhibitors as research tools and cancer drugs. 199(4), 583-588.
3. Stadtmueller BM and Hill CP (2011) Proteasome activators. Mol Cell 41(1):8-19.
4. Lasker K et al. (2012) Molecular architecture of the 26S proteasome holocomplex determined by an integrative approach. PNAS 109(5):1380-1387
5. Kisselev AF, et al. (2012) Proteasome inhibitors: an expanding army attacking a unique target. Chemistry &Biology, 19(1):99-115.
6. Kim Y-C and DeMartino GN (2011) C termini of proteasomal ATPases play nonequivalent roles in cellular assembly of mammalian 26 S proteasome. JBC 286(30): 26652–26666.