Lot # XXXXX

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MATERIAL DATA SHEET

S-100 Fraction Degradation Kit
Cat. # K-910

This kit contains reagents to allow for the controlled degradation of exogenously added or endogenously contained substrate proteins through the ubiquitin proteasome pathway (UPP). S-100 fraction contains the full complement of all UPP cytosolic enzymes and is ideal for the demonstration of a protein being targeted by the UPP for degradation.

NOTE: Please read this instruction material completely prior to performing the assay.

Product Information

Quantity/Stock:

1. **S100 *HeLa,*** 250 µL
   [S100] = 7.5 mg/ml in 50 mM HEPES pH 7.6, 1 mM DTT
2. **10 X Ubiquitin Solution**, 75 µL
   [Ub] = 50 mg/ml in 50 mM HEPES, pH 7.6
3. **10X Energy Regeneration Solution (ERS)**, 50 µL
4. **MG132 (Proteasome Inhibitor Control)**, 20 µL
   [MG132] = 200 µM in 100% DMSO

Storage: Store all solutions at -80°C. Avoid multiple freeze/thaw cycles.

Background

The Ubiquitin Proteasome System (UPS) is the cell's principle mechanism for protein catabolism. The UPS 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.

The proteasome is a large, multimeric protease that catalyzes the final step of UPS-mediated 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 26S Proteasome is defined by having one or two19S regulatory caps on the 20S core particle. It is the 26S that is responsible for the recognition and subsequent degradation of poly-ubiquitinated proteins into smaller polypeptides. This degradation is ubiquitin and ATP dependent and will not be 26S proteasome mediated unless target substrate is first ubiquitinated via the conjugation cascade.

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Technical Hints

The S100 fraction contains all the necessary components to conjugate ubiquitin to many substrate proteins, and also the components necessary for the degradation of these substrates by the proteasome. These enzymes/proteins include ubiquitin, E1, E2s, E3s (ligases), isopeptidases (UCHs) and proteasome (26S). Protein substrates of choice can be added to the S100 fraction, and then degradation may be analyzed via SDS-PAGE analysis. Tracking the degradation of the target protein depends on the detection method and user preference. Some examples include: 1) use of Western Blot analysis to visualize substrate degradation that is blocked in the presence of proteasome inhibitors, 2) use of SDS-PAGE and radiolabeled substrates to visualize substrate degradation that is blocked in the presence of proteasome inhibitors, and 3) generation of TCA-soluble radiolabeled peptides produced from proteasomal degradation of radiolabeled substrates.

If the chosen approach is to add a substrate protein and track the decrease of an SDS-PAGE band, care must be taken to add an amount of substrate that gives a clearly defined signal without the masking of the disappearance of the protein band which signifies degradation (i.e. overloading). Typical degradation rates will result in catabolism of 5-20% of the added substrate protein. If too much substrate is added, the gel signal will potentially hide the 5-20% reduction in substrate mentioned above. The optimal amount of substrate to be added to reactions must be determined empirically.

The following assay protocol is for the set-up and execution of the experimental proteasome degradation. Final substrate concentrations and incubation times must be determined experimentally for each individual protein. Detection methods must also be optimized based on activity, substrate quantity and time. Negative controls for demonstrating that substrate degradation is UPS-mediated include the omission of ERS, the addition of EDTA, or the addition of a proteasome inhibitor such as MG132 (included in the kit).

Reaction volumes may be scaled appropriately to suit individual needs. More information is available at techsupport@bostonbiochem.com.

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Recommended Assay Protocol (50 µl reaction)

1. Thaw all components by gently and continuously swirling tubes in a lukewarm water bath (≤ 30°C). Alternatively, tubes may be thawed with a rapid back-and-forth rolling motion between palms of hands. Do not heat tubes for an extended period of time. Once completely thawed, briefly centrifuge to collect contents at bottom of tubes and place tubes containing HeLa fraction, ERS solution, and ubiquitin on ice. Do not store MG132 on ice—it contains DMSO and will solidify if stored below room temperature. Wear gloves to prevent accidental exposure to DMSO.
2. For each 50 µl reaction that will be done, combine the following in order (mix after each addition):

    25 µl HeLa S-100 Fraction
    2 µl MG132 (***negative control only!*** Do not add inhibitor to degradation reactions. Allow negative control reactions to sit at room temperature for 15 minutes before continuing with further additions)
    5 µl 10X Ubiquitin Solution
    13 µl Substrate in desired buffer (user provided; suggested buffer is 50 mM HEPES pH 7.6, or similar)

3. Initiate conjugation/degradation reaction with the addition of 5 µl of ERS.
4. Mix gently and incubate at 37 °C for the desired incubation time. An initial time course is recommended to determine optimal activity and signal. Target degradation is often seen within 3-4 hours.
5. Depending on detection method, take appropriate quantity of the reaction at desired time and compare to proteasome inhibited negative control reaction. If observed target degradation is not inhibited in control reactions containing MG132, then the degradation is most likely NOT due to proteasomal degradation of the substrate.

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Literature

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