Lot # XXXXX

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MATERIAL DATA SHEET

S-100 Fraction Conjugation Kit
Cat. # K-915

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

NOTE: Please read this instruction material completely prior to performing the assay. This kit supplies enough materials for ten fifty-microliter reactions.

Product Information	
Quantity/Stock:	**1. S100** ***HeLa,*** 250 µl 7.5 mg/ml in 50 mM HEPES pH 7.6, 1 mM DTT **2. 10X Ubiquitin Solution**, 75 µl 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 200 µM in 100% DMSO **5. Ubiquitin Aldehyde,** 20 µg Lyophilized.
Storage:	Store all solutions at -80°C. Avoid multiple freeze/thaw cycles.

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Background

The Ubiquitin Proteasome System (UPS) is the cell's principle mechanism for regulated 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 covalent bond between ubiquitin and substrate proteins requires the activation of the C-terminal carboxyl group of ubiquitin to be activated. In this ATP-dependent reaction, ubiquitin is linked to the Ubiquitin Activating Enzyme (E1) through a thioester bond between its C-terminal glycine and the active site cysteine residue in the enzyme (E1-S-Ub). The activated ubiquitin is then transferred from E1 to one of a family of E2's (ubiquitin carrier enzymes) also utilizing a thioester linkage (E2-S-Ub). Finally, ubiquitin is transferred to the ε-amino group of lysine residues within substrate proteins in a process that requires the involvement of a third enzyme class, the ubiquitin ligases (E3). E3's bind to both specific substrate protein(s) and ubiquitin-charged E2's and facilitate the transfer of ubiquitin to the target in either a direct or indirect manner (depending on the E3 class). It is the formation of the E2-ubiquitin/E3/substrate complex that provides the exquisite specificity for the conjugation cascade.

Technical Hints

The S100 HeLa Fraction contains the necessary UPS components to ubiquitinate (and then potentially degrade) added protein substrates. These enzymes/proteins include ubiquitin, E1, E2s, many (but not all) E3 ligases, isopeptidases (UCHs) and the 26S proteasome. Protein substrates of choice can be added to the S100 fraction, and then results monitored using SDS-PAGE analysis. Tracking the ubiquitination of the target protein depends on the detection method and user preference. Some examples include: 1) use of SDS-PAGE + Western Blot analysis to visualize substrate ubiquitination, 2) use of SDS-PAGE and radiolabeled substrates to visualize substrate ubiquitination.

The following assay protocol is for the set-up and execution of the experimental proteasome conjugation. 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 may include omitting the addition of ERS, addition of EDTA, or the addition of thiol-modifying reagents such as N-Ethylmaleimide (NEM).

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 Solution 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. Reconstitute the vial of Ubiquitin-Aldehyde in 20 µl of 50 mM HCl. Alternately vortex and flick tube for 4-5 minutes so that contents are fully dissolved. Spin briefly in microfuge to collect contents and place tube on ice.
3. 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 (Note: Addition of proteasome inhibitor helps protect ubiquitinated substrates from proteasome-mediated degradation.)
    2 ul Ubiquitin Aldehyde Solution (Note: Addition of Ubiquitin Aldehyde helps protect ubiquitinated substrates from deubiquitinating enzymes, thereby leading to increased yield of ubiquitinated substrates in many instances. **Allow reactions to sit at room temperature for 15 minutes before continuing with further additions**)
    5 µl 10X Ubiquitin Solution
    11 µl Substrate in desired buffer (user provided; suggested buffer is 10-50 mM HEPES pH 7.6, or similar)

4. Initiate conjugation reaction with the addition of 5 µl of ERS.
5. Mix gently then centrifuge briefly to collect all contents to the bottom of the microfuge tube.
6. Incubate at 37 °C for the desired incubation time. An initial time course is recommended to determine optimal activity and signal. Target ubiquitination is often seen within 1-2 hours.

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Literature

References:

Driscoll J. and Goldberg A.L. (1990) J. Biol. Chem. **265**: 4789-4792
Ganoth D., *et al.* (1988) J. Biol. Chem. **263**: 12412-12419
Glickman M.H. and Ciechanover A. (2002) Physiol. Rev. **82**: 373-428
Hersko A. *et al.* (1983) J. Biol. Chem. **258**: 8206-8214
Waxman L., *et al.* (1987) J. Biol. Chem. **262**: 2451-2457
Voges D., *et al.* (1988) Ann. Rev, Biochem. **68**: 1015-1068

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