Lot # XXXXX

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MATERIAL DATA SHEET

Fraction II *HeLa* Degradation Kit
Cat. # K-920

This kit contains reagents to allow for the controlled degradation of exogenously added or endogenously contained substrate proteins through the ubiquitin proteasome pathway (UPP). Fraction II is the protein fraction of a cell extract that binds to anion exchange resin. It contains E1, most E2s, some E3s, UCHs, and the proteasome (20S and 26S); and is essentially free of ubiquitin and ATP.

NOTE: Kit contains reagents sufficient for 10 x 50 µl reactions.

Product Information	
Quantity/Stock:	**1. Fraction II *HeLa*,** 250 µL [FII] = X mg/ml in 50 mM HEPES, pH 7.6, 1 mM DTT. **2. Ubiquitin, 10 X stock**, 75 µL [Ub] = X mg/ml in 50 mM HEPES, pH 7.6 **3. Energy Regeneration Solution (ERS), 10X**, 50 µL **4. MG-132, Inhibitor Control**, 20 µL [MG-132] = X µM in 100% DMSO
Storage:	Enzyme/Fraction/Protein Solutions should be stored at -80°C. Avoid multiple freeze/thaw cycles.

Background

The Ubiquitin Proteasome Pathway (UPP) is the cell's principle mechanism for protein catabolism. The UPP 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 the UPP 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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Assay Considerations

Fraction II *HeLa* is the protein fraction of a cell extract that binds to anion exchange resin. It is essentially free of ubiquitin and ATP, and contains E1, most E2s, some E3s, UCHs, and the proteasome (20S and 26S). Protein substrate of choice can be added exogenously or if the protein of interest is already contained in the *HeLa* fraction, its degradation may be tracked via immuno-detection.

Tracking degradation of the target protein depends on the detection method and users preference. Below are some usual methods:

- Visualization of signal band on SDS-PAGE and subsequent disappearance that is blocked by the control proteasome inhibitor. Detection of the substrate band can be via radio-label or immuno-detection.
- Quantitative readout on the TCA soluble peptides produced over time that is blocked by the control proteasome inhibitor. This method is exclusively for radio-labeled substrates.

If adding substrate protein and tracking the decrease in a SDS-PAGE band is your approach, care must be taken to add substrate to an amount that gives a clearly defined signal without the masking of the disappearance of the protein band which signifies degradation. Typical degradation will catabolize 5-20% of the added substrate protein. If too much substrate is added, the gel signal will potentially hide the degradation of the 20% mentioned above. The ultimate optimal amount of substrate to be added must be determined experimentally. If the substrate protein is already contained in the *HeLa* fraction and is being assayed by immuno-detection, this is not an issue.

The following assay protocol is for the set-up and execution of the experimental proteasome degradation. Final concentrations of substrate and time courses must be determined experimentally for each individual protein. Detection methods must also be optimized based on activity, substrate quantity and time. The removal of ERS or the addition of inhibitor is a viable negative control for demonstration of a UPP mediated event.

Literature

References:

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