Lot #XXXXXX

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MATERIAL DATA SHEET

Deconjugating Enzyme FRET Substrate Kit
EDANS/DABCYL Labeled Substrates
Cat. # K-S18

Linkage specific diubiquitin is a substrate for enzymes that cleave the isopeptide linkage between two ubiquitin molecules. These FRET-based DUB substrates with isopeptide bonds between ubiquitin moieties are superior to gel densitometry assays. Since DUBs recognize and cleave substrates with specific steric conditions, each substrate varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

Product Information

Supplied:

EDANS/DABCYL FRET Substrates	Concentration	Quantity
1. K48 FRET Di-Ub Position 1 (EDANS)	X mg/ml (X μM)	10 μg
2. K48 FRET Di-Ub Position 2 (EDANS)	X mg/ml (X μM)	10 μg
3. K48 FRET Di-Ub Position 3 (EDANS)	X mg/ml (X μM)	10 μg
4. K63 FRET Di-Ub Position 1 (EDANS)	X mg/ml (X μM)	10 μg

Storage: Store at ≤ -70°C. Avoid multiple freeze/thaw cycles.

Stock: 50 mM Hepes pH 7.5, 150 mM NaCl, 2 mM DTT

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Background

These fluor-based substrates are superior to current stop time gel densitometry assays which are not scalable and are time and substrate consuming. However, DUB substrate specificity is influenced by many parameters and variables. These include but are not limited to: isopeptide linkages, fluor labels, tags and label/tag positions. Due to these variables, there is no universal diubiquitin substrate for all DUBs and variables must be optimized for each DUB. Each substrate in this kit varies in the position of the fluorophore and quencher and must be empirically tested for individual enzymes.

Below representative data is shown indicating the optimization of the label position on various substrates for USP5 (Isopeptidase T, E-322). Labeling at the same positions with alternative quench pairs (TAMRA/QXL) further improves efficiency and signal to background. Optimized quench pairs result in a more efficient substrate, a hydrolysis rate closer to wild-type ubiquitin and increased signal to background.

Assay Protocol Guidelines

The following protocol is based on typical concentrations for the USP5 mediated hydrolysis of diubiquitin substrates. Concentrations may vary depending on individual conditions and requirements. Below are suggested ranges of component final concentrations.

[Substrate] = 100-500 nM
[Enzyme] = 0.5-10 nM

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Sample Data

USP5 Mediated Hydrolysis of K48-linked (TAMRA) Substrates

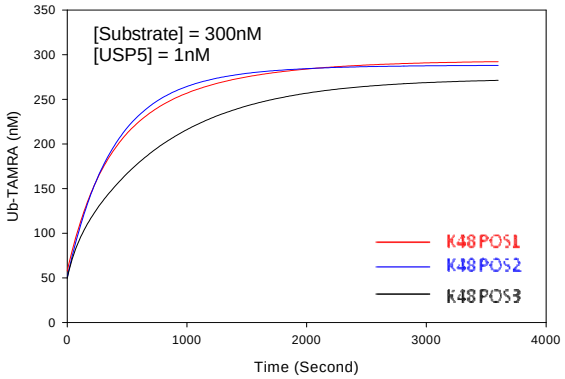

Figure 1. **USP5 catalyzed hydrolysis of K48 Di-Ub with different labeling sites (TAMRA/QXL570).** Substrates (300nM) incubated with 1nM USP5 50mM HEPES pH 7.5, 150mM NaCl, 2mM DTT. Hydrolysis was monitored at ex544nM, em572nM for 60 min.

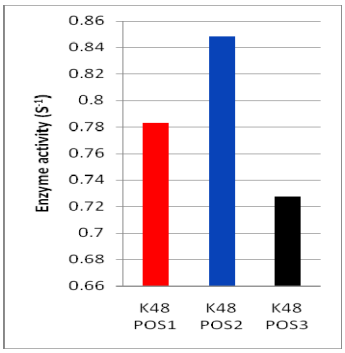

Figure 2. **USP5 activity comparison.** Specific activity of USP5 demonstrates label position dependent selectivity for K48-linked substrates.

USP5 Mediated Hydrolysis of K63-linked (TAMRA) Substrates

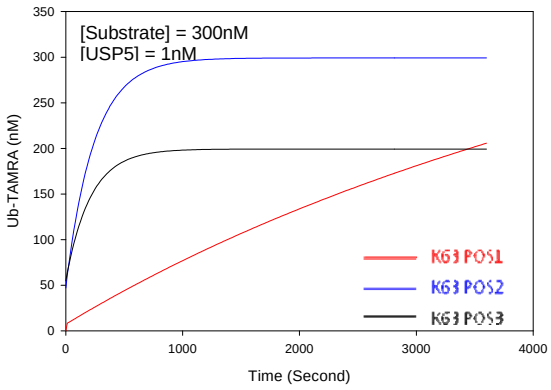

Figure 1. **USP5 catalyzed hydrolysis of K63 Di-Ub with different labeling sites (TAMRA/QXL570).** Substrates (300nM) incubated with 1nM USP5 50mM HEPES pH 7.5, 150mM NaCl, 2mM DTT. Hydrolysis was monitored at ex544nM, em572nM for 60 min.

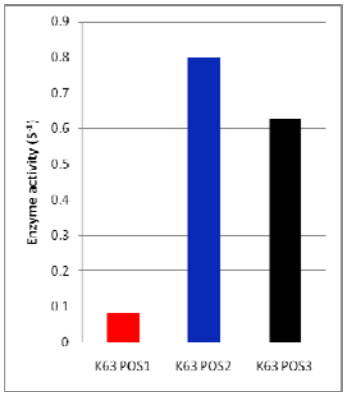

Figure 2. **USP5 activity comparison.** Specific activity of USP5 demonstrates label position dependent selectivity for K63-linked substrates.

USP5 Mediated Hydrolysis of K11-linked (TAMRA) Substrates

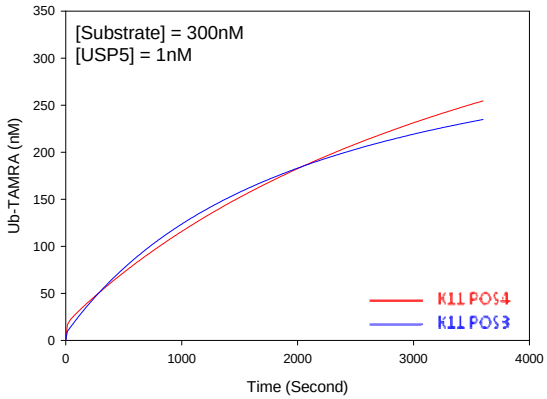

Figure 1. **USP5 catalyzed hydrolysis of K11 Di-Ub with different labeling sites (TAMRA/QXL570).** Substrates (300nM) incubated with 1nM USP5 50mM HEPES pH 7.5, 150mM NaCl, 2mM DTT. The hydrolysis was monitored at ex544nM, em572nM for 60 min.

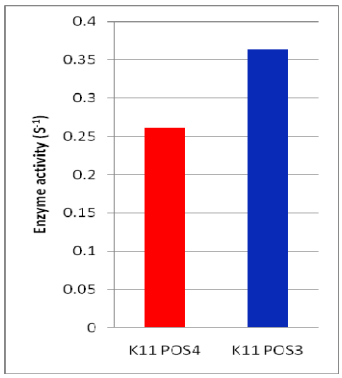

Figure 2. **USP5 activity comparison.** Specific activity of USP5 demonstrates label position dependent selectivity for K11-linked substrates.

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Literature

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