Lot # XXXXX

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MATERIAL DATA SHEET

ISG15 Conjugation Kit
Cat. # K-600

This kit is designed for the conjugation of the ubiquitin-like modifier ISG15 to protein substrates *in vitro*, which requires the activities of the human ISG15 E1 activating enzyme and the UbcH8 E2 enzyme. The E1 enzyme charges the ISG15 by forming an ATP-dependent high energy thioester bond. The activated ISG15 is subsequently transferred to UbcH8 and this E2-S-ISG15 thioester complex can be used for the conjugation of ISG15 to protein substrates with the addition of necessary E3 enzymes (not supplied).

NOTE: Kit contains reagents sufficient for 10 x 20 µl reactions.

Product Information

		Concentration	Volume
Supplied:	**1. 10X ISG15 E1 Enzyme**	X mg/ml (X µM)	20 µl
	2. 5X ISG15	X mg/ml (X µM)	40 µl
	3. 10X UbcH8	X mg/ml (X µM)	20 µl
	4. 10X Mg-ATP Solution	X mM	20 µl
	5. 10X Reaction Buffer	X mM Hepes pH 8.0 X mM NaCl	20 µl

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

Background

The ubiquitin-like ISG15 is conjugated to a variety of proteins in the presence of UbcH8 and an E1 activating enzyme. The ISG15 E1 enzyme uses ATP to adenylate the C-terminal glycine residue of ISG15, forming a high-energy thioester bond. The second step is the trans-esterification reaction whereby the activated ISG15 is transferred to the active site cysteine of UbcH8. UbcH8 is a member of the E2 family and is homologous to ubiquitin-conjugating enzymes, but is specific for the conjugation of ISG15 to a variety of target proteins. The ISG15 pathway is distinct from ubiquitination with different substrate specificity and interaction with ligating enzymes. ISG15 becomes conjugated to a diverse set of proteins after IFN-α/β stimulation or microbial challenge. The functions or biochemical consequences ISG15 conjugation to proteins are not yet known, but it appears that this modification does not target proteins for proteasomal degradation. ISG15 shows specific chemotactic activity towards neutrophils and activates them to induce release of eosinophil chemotactic factors. It may also serve as a trans-acting binding factor directing the association of ligated target proteins to intermediate filaments; and may also be involved in autocrine, paracrine and endocrine mechanisms.

840 Memorial Drive, Cambridge, MA 02139 Phone: 617-241-7072 FAX: 617-492-3565
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Assay Protocol

The following protocol is based on typical concentrations for the thioester reaction. Concentrations may be varied depending on individual conditions and requirements. Below are the suggested ranges of component final concentrations.

$E1^{ISG15}$ enzyme: 100-500 nM
ISG15: 10-100 μM
UbcH8: 5-15 μM

Recommended Assay Protocol

Protocol (20 μL reaction):

1. Add 2 μl $E1^{ISG15}$ enzyme (250 nM final).
2. Add 4 μl ISG15 protein (20 μM final).
3. Add 2 μl reaction buffer (1X final).
4. Add 2 μl UbcH8 enzyme (10 μM final).
5. Add any other desired components, such as substrate of interest. The final concentration of the substrate should be determined experimentally by titration.
6. Bring the volume to a total of 18 μL with dH2O if needed.
7. Initiate reaction with the addition of 2 μL of Mg-ATP solution (1 mM final).
8. Incubate the reaction at 37°C for 30-60 minutes. An initial time course is recommended to determine the optimal incubation time for efficient substrate conjugation. Depending on experimental conditions, the incubation time required might be greater than 60 minutes.
9. Useful negative controls to include are: running the reaction in the absence of ATP, in the presence of 1 mM EDTA or in the presence of 5 mM DTT.

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Literature

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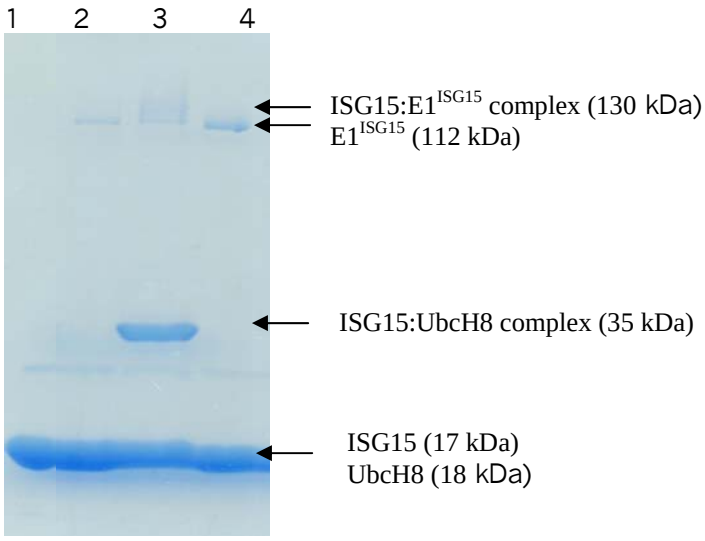

Figure 1. ISG15:UbcH8 Thiolester Complex Formation.
Reactions (100 nM ISG15 Activating Enzyme ($E1^{ISG15}$), 5 μM UbcH8, 50 μM ISG15 in 50 mM Hepes pH 8, 50 mM NaCl) were incubated at 37°C for 30 minutes and subjected to SDS-PAGE under non-reducing conditions. Lane 1: ISG15 only. Lanes 2 and 3: $E1^{ISG15}$, 5 μM UbcH8, 50 μM ISG15 in the absence and presence of 10 mM Mg-ATP respectively. Lane 4: $E1^{ISG15}$, 5 μM UbcH8, 50 μM ISG15, 10 mM Mg-ATP and 2 mM DTT.

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