

MATERIAL DATA SHEET

ISG15 Conjugation Initiation 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			
		<u>Concentration</u>	<u>Volume</u>
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	500 mM Hepes pH 8.0 1000 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.

Literature

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