Lot # XXXXX

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MATERIAL DATA SHEET

Parkin Auto-Ubiquitination Kit
Cat. # K-105

This kit is designed as a control for to the conjugation of the ubiquitin to protein substrates *in vitro*, which requires the activities of the ubiquitin E1 (**E-305**), E2 UbcH7 (**E2-640**) and E3 Parkin (**E3-150**) activating enzyme. The E1 enzyme charges the ubiquitin by forming an ATP-dependent high energy thiolester bond. The activated ubiquitin is subsequently transferred to UbcH7 then to Parkin. The Parkin-S-Ub complex has the ability to both auto-ubiquitinate and transfer the ubiquitin to various substrates such as CDCrel-1 and rel-2a, cyclin E, synphilin-1, the O-glycosylated form of α-synuclein (αSp22), PAel-R, FBP1, α/β tubulin, RanBP2, Hsp70, synaptotagmin XI. Other E2s that have shown activity with Parkin are UbcH8 (**E2-644**) and UbcH13/Uve1a (**E2-664**). Alternatively labeled ubiquitins may be substituted for biotin-ubiquitin for visualization such as fluorescein-ubiquitin (**U-590**) and rhodamine-ubiquitin (**U-600**).

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

Concentration of components vary with Lot #.

Product Information

Supplied:		**Concentration**	**Volume**
1.	**10X E1 Enzyme**	X mg/ml (X µM)	20 µl
2.	**10X UbcH7**	X mg/ml (X µM)	20 µl
3.	**10X His_6-Parkin**	X mg/ml (X µM)	20 µl
4.	**10X Biotin-Ubiquitin**	X mM	20 µl
5.	**10X Reaction Buffer**	X mM	20 µl

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

Background

Mutations in the Parkin (PRKN2) gene are considered to be a major cause of autosomal recessive juvenile parkinsonism (AR-PJ). Parkin functions as an E3 ligase having an N-terminal ubiquitin-like motif and a C-terminal RING domain composed of two RING finger motifs separated by two IBR domains. Parkin can auto-ubiquitination itself and ubiquitinate various substrates (eg. CDCrel-1 and rel-2a, cyclin E, synphilin-1, the O-glycosylated form of α-synuclein (αSp22), PAel-R, FBP1, α/β tubulin, RanBP2, Hsp70, synaptotagmin XI) in an E2-dependent manner, targeting them for degradation. Parkin functions in conjunction E2 enzymes UbcH7 (**E2-640**), UbcH8 (**E2-644**) and UbcH13/Uev1 (**E2-664**). Parkin disease-associated mutations often affect E3 ligase activity through decreased E2 and/or substrate interactions which may thus result in the dysfunction of proteasomal degradation pathways and the neurotoxic accumulation of misfolded proteins.

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Literature

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