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Certificate of Analysis

Parkin (c-Myc tagged), active

(Recombinant E3 ligase expressed in Sf21 insect cells)
Item # 23-046, 23-046-K, 23-046M
Parent Lot # D11PP050N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: *N*-terminal c-Myc, 6His-tagged, recombinant human Parkin full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.
Purity 94% by SDS-PAGE and Coomassie blue staining. MW = 57kDa.

Activity (Parent lot# D11PP050N): This lot of Parkin (c-Myc tagged), active is active and meets product specifications.

Formulation: 2.149mg/ml of enzyme in a buffer containing 50mM Tris/HCl pH7.5, 150mM NaCl, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

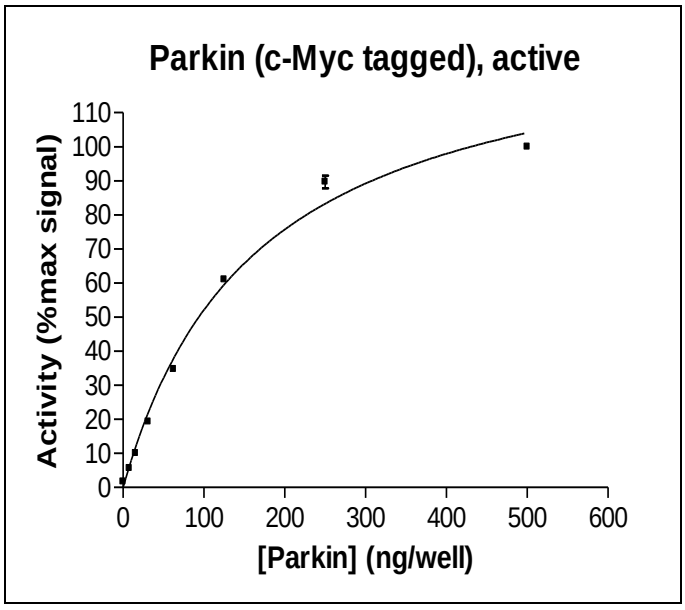
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

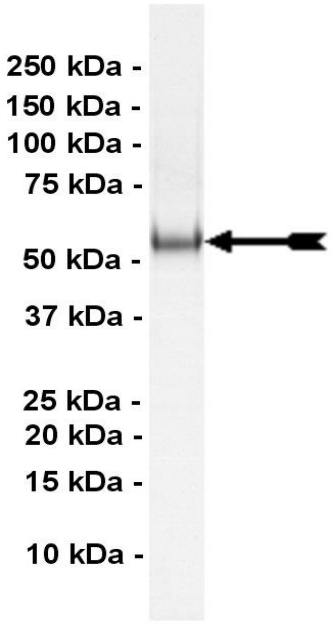
Quality Control Testing

Assay: This enzyme was titrated in a ubiquitination assay and the results normalised against the maximum signal.



Protein Identity: Confirmed identity as Parkin by mass spectrometry.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Parkin, active.



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E3 Assay Protocol

Reagents:

1. **UBE1, active (Item # 23-021)**
2. **UbcH7, active (Item # 23-047)**
3. **Parkin (c-Myc tagged), active (Item # 23-046)**
4. **1x Reaction Buffer**
5. **Biotinylated-Ubiquitin**
6. **Stop Solution**

Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$).

Parkin (c-Myc tagged), active is incubated with 25mM MOPS pH 7.5, 0.01% Tween 20, 5mM $MgCl_2$, 10µM ATP, 10nM UBE1, 500nM UbcH7 and 2µM biotinylated-ubiquitin. The reaction is initiated with the addition of biotinylated-ubiquitin. After 30 minutes at room temperature the reaction is terminated by the addition of 25mM MOPS pH7.5 containing 125mM EDTA, 150mM NaCl, and 0.05% Tween 20. Reaction products are separated by capture onto a microplate coated with anti-c-Myc antibody and washing with PBS containing 0.05% Tween 20. Parkin activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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Parkin Information

Protein human Parkin

Accession number GenBank NM_004562

Alternative Names Parkinson juvenile disease protein 2, PARK2, PRKN

Key Facts Parkin is an E3-ubiquitin ligase belonging to the RBR (RING – In Between RING – RING) family. Mutations in Parkin that result in a loss-of-function are the most frequent cause of familial Parkinson's disease and are responsible for a large percentage of autosomal recessive juvenile parkinsonism (AR-JP). In addition to its auto-ubiquitination, Parkin can also ubiquitinate various substrates, including the CDK2-interacting protein cyclin E1, the α-synuclein-interacting protein synphilin-1, the synaptic vesicle-associated CDC-rel1 and the p38 subunit of aminoacyl-tRNA synthetase/JTV-1. Ubiquitination of substrates occurs in conjunction with the E2 enzymes UbcH7, UbcH8 or UbcH13/Uev1.

Related Products Item # 23-021 UBE1, active, Item # 23-047 UbcH7, active

Selected References

Zhang Y. *et al*., Parkin Functions as an E2-Dependent Ubiquitin–Protein Ligase and Promotes the Degradation of the Synaptic Vesicle-Associated Protein, CDCrel-1. Proc Nat Acad Sci., *97*: 13354–13359, 2000

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Imai Y. *et al*., Parkin Suppresses Unfolded Protein Stress-Induced Cell Death through Its E3 Ubiquitin Protein Ligase Activity. J. Biol Chem., *275*: 35661-35664, 2000

Dev K.K. *et al.*, Parkin-Associated Proteins and Parkinson's Disease. Neuropharmacology, *45*: 1-13, 2003

Hampe C. *et al*., Biochemical Analysis of Parkinson's Disease-Causing Variants of Parkin, an E3 Ubiquitin–Protein Ligase with Monoubiquitylation Capacity. Human Molecular Genetics,*15*: 2059-2075, 2006

Reviewed and approved by site quality representative.