

Certificate of Analysis

Itk, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-660, 14-660-K, 14-660M

Parent Lot # 32868U

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 6His-tagged recombinant, human Itk, amino acids 352–617, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 95% by SDS-PAGE and Coomassie blue staining. MW = 34kDa.

Specific Activity (Parent lot# 32868U): 101U/mg, where one unit of Itk activity is defined as 1nmol phosphate incorporated into 0.333mg/ml myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.15mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 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.

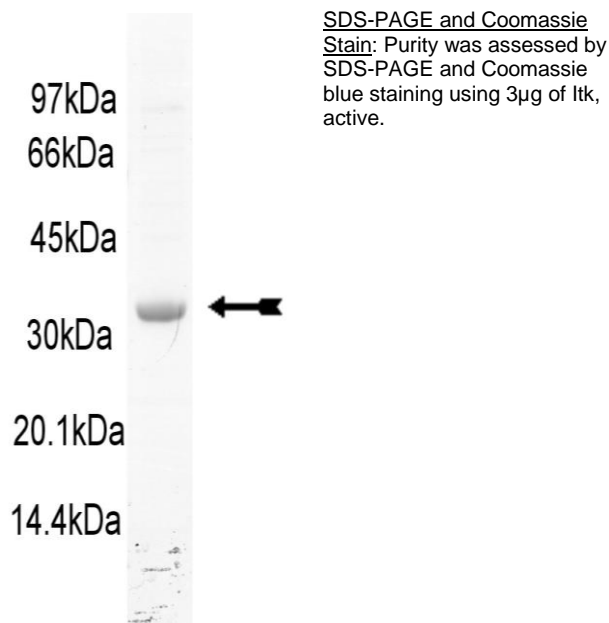
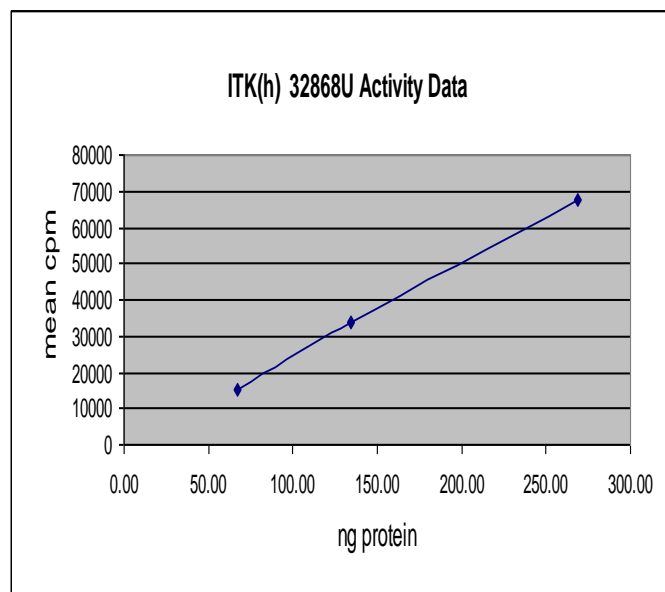
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

Kinase Assay: 67–268ng of this lot of enzyme phosphorylated 0.333mg/ml myelin basic protein (MBP) in the assay described on page two.

MS Tryptic Fingerprint: Confirmed identity as Itk with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS-NaOH pH7.0, 1mM EDTA.
2. **Myelin Basic Protein (MBP):** Use at a final concentration of 0.333mg/ml. Make a 3.33mg/ml stock. Add 2.5µl of stock per assay point.
3. **Itk, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 67–268ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **myelin basic protein (MBP)**.
3. Add **2.5µl (67–268ng) Itk, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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Itk Sequence Information

<u>Protein</u>	Human Itk
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	R29 of the recombinant protein is equivalent to R352 of human Itk
<u>Accession number</u>	GenBank NM_005546

Recombinant Itk amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMGSR YGKWVIDPSEL TFVQEIGSGQ FGLVHLGYWL
61 NKDKVAIKTI REGAMSEEDF IEEAEVMMKL SHPKLVQLYG VCLEQAPICL VFEFMEHGCL
121 SDYLRTQRGL FAAETLLGMC LDVCEGMAYL EEACVIHRDL AARNCLVGEN QVIKVSDFGM
181 TRFVLDDQYT SSTGTFKFPVK WASPEVFSFS RYSSKSDVWS FGVLMWEVFS EGKIPYENRS
241 NSEVVEDIST GFRLYKPRLA STHVYQIMNH CWKERPEDRP AFSRLLRQLA EIAE

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Recombinant Itk nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg ggcggcatggg atccagatac gggaaatggg tgatcgacct ctcagagctc
121 actttttgtgc aagagattgg cagtgggcaa tttgggttgg tgcattctggg ctactggctc
181 aacaaggaca aggtggctat caaaaccatt cggaaggagg ctatgtcaga agaggacttc
241 atagaggagg ctgaagtaat gatgaaactc tctcatccca aactgggtgca gctgtatggg
301 gtgtgcctgg agcaggcccc catctgcctg gtgtttgagt tcatggagca cggctgcctg
361 tcagattatc tacgcaccca gcggggactt tttgctgcag agacctgctt gggcatgtgt
421 ctggatgtgt gtgagggcat ggcctacctg gaagaggcat gtgtcatcca cagagacttg
481 gctgccagaa attgtttggt gggagaaaac caagtcatca aggtgtctga ctttgggatg
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601 tgggcatccc cagaggtttt ctctttcagt cgctatagca gcaagtccga tgtgtggtca
661 tttggtgtgc tgatgtggga agttttcagt gaaggcaaaa tcccgtatga aaaccgaagc
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841 gccttctcca gactgctgcg tcaactggct gaaattgcag aatag

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Reviewed and approved by site quality representative.

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