

**Discovery Services** 

## **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 ltk, amino acids 352–617, expressed by baculovirus in Sf21 insect cells. Purified using Ni<sup>2+</sup>/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 microcentrifuge 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**

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



<u>MS Tryptic Fingerprint</u>: 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.
- 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.
- [γ-<sup>33</sup>P]ATP: 2.5 x magnesium acetate/[γ-<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-<sup>33</sup>P]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) ltk, active.
- 4. Add 5µl of dH<sub>2</sub>O.
- 5. Add 10µl of diluted [ $\gamma$ -<sup>33</sup>P]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
Tags	N-terminal 6His
Native sequence	R29 of the recombinant protein is equivalent to R352 of human Itk
Accession number	GenBank NM_005546

#### Recombinant Itk amino acid sequence:

1	МЗҮҮННННН	DYDIPTTENL	YFQGAMGSRY	GKWVIDPSEL	TFVQEIGSGQ	FGLVHLGYWL
61	NKDKVAIKTI	REGAMSEEDF	IEEAEVMMKL	SHPKLVQLYG	VCLEQAPICL	VFEFMEHGCL
121	SDYLRTQRGL	FAAETLLGMC	LDVCEGMAYL	EEACVIHRDL	AARNCLVGEN	QVIKVSDFGM
181	TRFVLDDQYT	SSTGTKFPVK	WASPEVFSFS	RYSSKSDVWS	FGVLMWEVFS	EGKIPYENRS
241	NSEVVEDIST	GFRLYKPRLA	STHVYQIMNH	CWKERPEDRP	AFSRLLRQLA	EIAE

#### Recombinant Itk nucleotide sequence:

1	atgtcgtact	accatcacca	tcaccatcac	gattacgata	tcccaacgac	cgaaaacctg
61	tattttcagg	gcgccatggg	atccagatac	gggaaatggg	tgatcgaccc	ctcagagctc
121	acttttgtgc	aagagattgg	cagtgggcaa	tttgggttgg	tgcatctggg	ctactggctc
181	aacaaggaca	aggtggctat	caaaaccatt	cgggaagggg	ctatgtcaga	agaggacttc
241	atagaggagg	ctgaagtaat	gatgaaactc	tctcatccca	aactggtgca	gctgtatggg
301	gtgtgcctgg	agcaggcccc	catctgcctg	gtgtttgagt	tcatggagca	cggctgcctg
361	tcagattatc	tacgcaccca	gcggggactt	tttgctgcag	agaccctgct	gggcatgtgt
421	ctggatgtgt	gtgagggcat	ggcctacctg	gaagaggcat	gtgtcatcca	cagagacttg
481	gctgccagaa	attgtttggt	gggagaaaac	caagtcatca	aggtgtctga	ctttgggatg
541	acaaggttcg	ttctggatga	tcagtacacc	agttccacag	gcaccaaatt	cccggtgaag
601	tgggcatccc	cagaggtttt	ctctttcagt	cgctatagca	gcaagtccga	tgtgtggtca
661	tttggtgtgc	tgatgtggga	agttttcagt	gaaggcaaaa	tcccgtatga	aaaccgaagc
721	aactcagagg	tggtggaaga	catcagtacc	ggatttcggt	tgtacaagcc	ccggctggcc
781	tccacacacg	tctaccagat	tatgaatcac	tgctggaaag	agagaccaga	agatcggcca
841	gccttctcca	gactgctgcg	tcaactggct	gaaattgcag	aatag	

#### Reviewed and approved by site quality representative.

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