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Certificate of Analysis

IRR, active

(Recombinant enzyme expressed in *Sf21* insect cells)
Item # 14-645, 14-645-K, 14-645M
Parent Lot # 1980269

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 IRR, amino acids 944–1266 expressed by baculovirus in *Sf21* insect cells. Purified using Ni^{2+}-NTA agarose. Purity 71.2% by SDS PAGE and Coomassie blue staining. MW = 37.9kDa.

Specific Activity (Parent lot# 1980269): 42U/mg, where one unit of IRR, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.566mg/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.

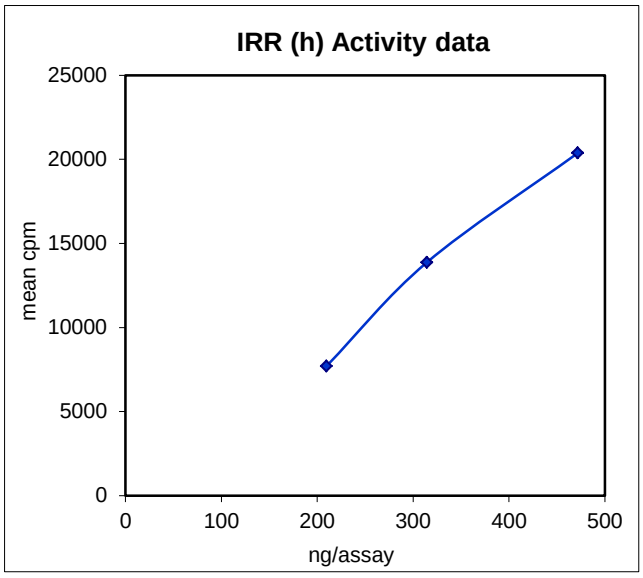
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

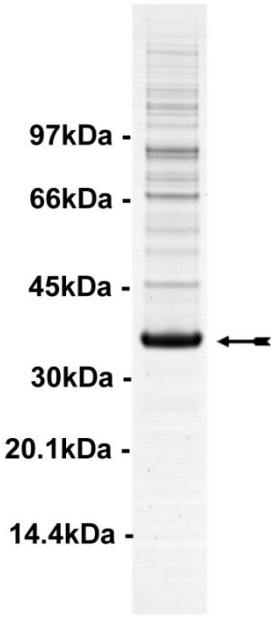
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 210–472ng of this lot of enzyme phosphorylated 0.33mg/ml myelin basic protein (MBP) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



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



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

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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 assay concentration of 0.33mg/ml. Make up a 3.3mg/ml stock. Use 2.5µl of stock per assay point.
3. **IRR, active:** Dilute with 20mM MOPS/NaOH pH 7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 210–472ng per assay point.
4. **[γ-^{33}P]ATP**: 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}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 (210–472ng) IRR, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}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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IRR Sequence Information

Protein human IRR

Tags *N*-terminal 6His

Native sequence F9 of recombinant sequence is equivalent to F943 of native human IRR

Accession number GenBank NM_014215

Recombinant IRR amino acid sequence:

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  1 MHHHHHHEFY GKKRNRTLYA SVNPEYFSAS DMYVPDEWEV PREQISIIRE LGQGSFGMVY
 61 EGLARGLEAG EESTPVALKT VNELASPREC IEFLKEASVM KAFKCHHVVR LLGVVSQGQP
121 TLVIMELMTR GDLKSHLRSL RPEAENNPGL PQPALGEMIQ MAGEIADGMA YLAANKFVHR
181 DLAARNCMVS QDFTVKIGDF GMTRDVYETD YYRKGGKGLL PVRWMAPESL KDGIFTTHSD
241 VWSFGVVLWE IVTLAEQPYQ GLSNEQVLKF VMDGGVLEEL EGCPLQLQEL MSRCWQPNPR
301 LRPSFTHILD SIQEELRPSF RLLSFYYSPE CR
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Recombinant IRR nucleotide sequence:

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  1 atgcatcatc accatcacca tgaattctac ggcaagaaga gaaacagaac cctgtatgct
 61 tctgtgaatc cagagtactt cagcgcctct gatatgtatg tccctgatga atgggaggtg
121 cctcgggagc agatctcgat aatccgggaa ctgggccagg gctcttttgg gatggtatat
181 gaggggctgg cacgaggact tgaggctgga gaggagtcca cacccgtggc cctgaagacg
241 gtgaatgagc tggccagccc acgggaatgc attgagttcc tcaaggaagc ttctgtcatg
301 aaagccttca agtgtcacca tgtggtgcgt ctcctgggtg tggtatctca gggccagcca
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421 cggcctgagg cagagaacaa ccctgggctc ccacagccag cattgggggа aatgatccaa
481 atggctggtg agattgcaga cggcatggcc taccttgctg ccaacaagtt tgtgcaccga
541 gatctagcag cccgcaactg catggtgtcc caggacttca ccgtcaagat cggggacttc
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Reviewed and approved by site quality representative.