Product Information

FGR, active, GST-tagged, human
PRECISIO® Kinase
recombinant, expressed in Sf9 cells

Catalog Number F8055
Storage Temperature –70 °C

Synonyms: SRC2, c-fgr, p55c-fgr

Product Description
FGR is a protooncogene that is a unique member of the tyrosine kinase gene family. Certain lymphomas, but not sarcomas nor carcinomas, express FGR-related messenger RNA. This transcript is detected in Burkitt's lymphoma cell lines that are naturally infected with Epstein-Barr virus (EBV), but not in EBV-negative Burkitt's lymphoma cells.\(^1\) FGR expression is limited to normal peripheral blood granulocytes, monocytes, and alveolar macrophages, all of which contain 50–100 copies of c-fgr mRNA per cell.\(^2\)

This recombinant product was expressed by baculovirus in Sf9 insect cells using an N-terminal GST-tag. The gene accession number is NM 005248. It is supplied in 50 mM Tris-HCl, pH 7.5, with 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~86 kDa

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot:
≥70% (SDS-PAGE, densitometry)

Figure 2.
Specific Activity of Typical Lot:
242–328 nmole/min/mg

Procedure
Preparation Instructions
Kinase Assay Buffer – 25 mM MOPS, pH 7.2, 12.5 mM glycerol 2-phosphate, 25 mM MgCl₂, 5 mM EGTA, and 2 mM EDTA. Just prior to use, add DTT to a final concentration of 0.25 mM.

Kinase Dilution Buffer – Dilute the Kinase Assay Buffer 5-fold with a 50 ng/µl BSA solution.
Kinase Solution – Dilute the active FGR (0.1 µg/µl) with Kinase Dilution Buffer to the desired concentration. **Note:** The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active FGR kinase for optimal results.

10 mM ATP Stock Solution – Dissolve 55 mg of ATP in 10 ml of Kinase Assay Buffer. Store in 200 µl aliquots at –20 °C.

γ-32P-ATP Assay Cocktail (250 µM) – Combine 5.75 ml of Kinase Assay Buffer, 150 µl of 10 mM ATP Stock Solution, 100 µl of γ-32P-ATP (1 mCi/100 µl). Store in 1 ml aliquots at –20 °C.

Substrate Solution – Dissolve the synthetic peptide substrate Poly (Glu:Tyr, 4:1) in water at a final concentration of 1 mg/ml.

1% phosphoric acid solution – Dilute 10 ml of concentrated phosphoric acid to a final volume of 1 L with water.

**Kinase Assay**

This assay involves the use of the 32P radioisotope. All institutional guidelines regarding the use of radioisotopes should be followed.

1. Thaw the active FGR, Kinase Assay Buffer, Substrate Solution, and Kinase Dilution Buffer on ice. The γ-32P-ATP Assay Cocktail may be thawed at room temperature.
2. In a pre-cooled microcentrifuge tube, add the following solutions to a volume of 20 µl:
   - 10 µl of Kinase Solution
   - 10 µl of Substrate Solution
3. Set up a blank control as outlined in step 2, substituting 10 µl of cold water (4 °C) for the Substrate Solution.
4. Initiate each reaction with the addition of 5 µl of the γ-32P-ATP Assay Cocktail, bringing the final reaction volume to 25 µl. Incubate the mixture in a water bath at 30 °C for 15 minutes.
5. After the 15 minute incubation, stop the reaction by spotting 20 µl of the reaction mixture onto an individually precut strip of phosphocellulose P81 paper.
6. Air dry the precut P81 strip and sequentially wash in the 1% phosphoric acid solution with constant gentle stirring. It is recommended the strips be washed a total of 3 times of ~10 minutes each.
7. Set up a radioactive control to measure the total γ-32P-ATP counts introduced into the reaction. Spot 5 µl of the γ-32P-ATP Assay Cocktail on a precut P81 strip. Dry the sample for 2 minutes and read the counts. Do not wash this sample.
8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
9. Determine the corrected cpm by subtracting the blank control value (see step 3) from each sample and calculate the kinase specific activity

**Calculations:**

1. Specific Radioactivity (SR) of ATP (cpm/nmole)

\[
SR = \frac{\text{cpm of 5 µl of γ-32P-ATP Assay Cocktail}}{\text{nmole of ATP}}
\]

   \[\text{cpm – value from control (step 7)}\]

   \[\text{nmole – 1.25 nmole (5 µl of 250 µM ATP Assay Cocktail)}\]

2. Specific Kinase Activity (SA) (nmole/min/mg)

\[
\text{nmole/min/mg} = E \times T \\
\text{SR} = \frac{\Delta \text{cpm} \times (25/20)}{\text{SR} \times E \times T}
\]

   \[\text{SR} = \text{specific radioactivity of the ATP (cpm/nmole ATP)}\]

   \[\Delta \text{cpm} = \text{cpm of the sample – cpm of the blank (step 3)}\]

   \[25 = \text{total reaction volume}\]

   \[20 = \text{spot volume}\]

   \[T = \text{reaction time (minutes)}\]

   \[E = \text{amount of enzyme (mg)}\]

**References**


PRECISIO is a registered trademark of Sigma-Aldrich Co. LLC.

©2014 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA-ALDRICH is a trademark of Sigma-Aldrich Co. LLC, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.