Recombinant Human FGFR2 kinase domain His-tag

Catalog Number: 11812-FR

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived human FGFR2 protein Met459-Leu772, with a N-terminal 6-His tag Accession # P21802.1
N-terminal Sequence Analysis	Protein identity confirmed by mass spectrometry
Predicted Molecular Mass	37 kDa

SPECIFICATIONS

SDS-PAGE	35-39 kDa, under reducing conditions.
Activity	Measured by its ability to transfer phosphate from adenosine triphosphate (ATP) to a peptide substrate. The specific activity is >75 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>80%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 20 mM $MgCl_2$, 5 mM $MnCl_2$, 0.1 mg/mL BSA, pH 7.5
- Recombinant Human FGFR2 kinase domain His-tag (rhFGFR2) (Catalog # 11812-FR)
- Poly (4:1 Glu, Tyr) Peptide, 1 mg/mL stock in 25 mM Tris, pH 7.5
- Adenosine Triphosphate (ATP), 10 mM stock in deionized water
- ADP-Glo™ Kinase Assay Kit (Promega)
- White 96-well Plate
- Plate Reader with Luminescence Read Capability

Assay

1. Dilute rhFGFR2 to 10 µg/mL in Assay Buffer.
2. Prepare Substrate Mixture containing 200 µM ATP and 0.6 mg/mL Poly (4:1 Glu, Tyr) Peptide in Assay Buffer.
3. Combine equal volumes of 10 µg/mL rhFGFR2 and Substrate Mixture. Create a Substrate Control containing equal volumes of Assay Buffer and Substrate Mixture.
4. Incubate at room temperature for 40 minutes in the dark.
5. After incubation, transfer 10 µL of each reaction to a plate.
6. Terminate the reaction and deplete the remaining ATP by adding 10 µL of ADP-Glo™ Reagent (supplied in kit) to all wells.
7. Incubate at room temperature for 40 minutes in the dark.
8. Add 20 µL Kinase Detection Reagent (supplied in kit) to all wells.
9. Incubate at room temperature for 30 minutes in the dark.
10. Read plate in Luminescence endpoint mode.
11. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Luminescence* (RLU) x Conversion Factor** (pmol/RLU)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Control

**Derived from ADP-Glo™ Kinase Assay Kit protocol (Promega)

Final Assay Conditions

Per Reaction:

- rhFGFR2: 5 µg/mL
- ATP: 100 µM
- Poly (4:1 Glu, Tyr) Peptide: 0.3 mg/mL

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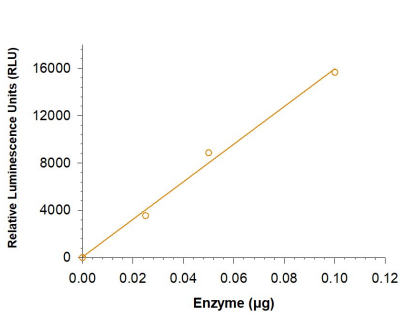
PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

Enzyme Activity



Recombinant Human FGFR2 kinase domain His-tag Enzyme Activity. Recombinant Human FGFR2 kinase domain His-tag (Catalog # 11812-FR) is measured by its ability to transfer phosphate from adenosine triphosphate (ATP) to a peptide substrate.

BACKGROUND

Fibroblast growth factor receptor 2 (FGFR2), also known as Keratinocyte growth factor receptor (KGFR), is one of four highly conserved tyrosine protein kinase FGFR receptors within the tyrosine protein kinase family that differ in ligand affinity and tissue distribution. FGFR2, like other receptor members in this family, is comprised of an extracellular domain (ECD) with immunoglobulin-like segments that binds its target cytokine FGF and heparin sulfate to induce dimerization and autophosphorylation, a single-pass transmembrane domain, and a cytosolic kinase domain that triggers downstream signaling cascades through multiple pathways (1, 2). Like the structure of other tyrosine kinases, the kinase domain contains the conserved catalytic domain with an ATP binding site within the cleft of two lobes at the N-terminal and C-terminal regions as well as an activation loop at the surface between the two lobes (3). FGFR2 has two naturally occurring isoforms, FGFR2b and FGFR2c, that are created by splicing of the third immunoglobulin-like domain and lead to tissue specific expression within epithelial and mesenchyme derived tissues respectively to bind different FGFs (4, 5). Missense mutations of FGFR2 that cause gain of function cause several congenital skeletal disorders including Apert, Crouzon, and Pfeiffer syndromes (6, 7). In addition, FGFR2 regulates signaling pathways that promote cell proliferation, inhibition of apoptosis, and angiogenesis (5). FGFR2 has been reported to be overexpressed or mutated in many tumors including brain, breast, cholangiocarcinoma, gastric, lung, ovarian and uterine, some of which have been further correlated with poor prognosis (5, 8-15). Inhibition of FGFR2 has been identified as a promising cancer therapeutic strategy and there is significant ongoing research in the development of drugs that inhibit FGFR2 using multikinase and more selective ATP-binding site small molecule and antibody drugs (2, 5, 14, 16).

References:

1. Hubbard, S.R. and J.H. Till (2000) *Annu. Rev. Biochem.* **69**:373.
2. Katoh, M. *et al.* (2024) *Nature Rev. Clin. Oncol.* **21**:312.
3. Dai, S. *et al.* (2019) *Cell.* **8**:614.
4. Orr-Urtreger A. *et al.* (1993) *Developmental Biology.* **158**:475.
5. Lages Dos Santos,J. *et al.* (2025) *Medicina* **61**:1890.
6. Pollock, P.M. *et al.* (2007) *Oncogene* **26**:7158.
7. Tuzon, C.T. *et al.* (2019) *Curr. Osteoporos Rep.* **17**:138.
8. Shoji H. *et al.* (2015) *Anticancer Res.* **35**:5055.
9. Liu, G. *et al.* (2017) *Tumour Biol.* **39**:1010428317707424.
10. Mahipal, A. *et al.* (2019) *Cancer Treat. Rev.* **78**:1.
11. Georgescu, M.M. *et al.* (2021) *Acta Neuropathol. Commun.* **9**:69.
12. Jogo, T. *et al.* (2021) *Clin. Cancer Res.* **27**:5619.
13. Pacini, L. *et al.* (2021) *Cells* **10**:1154.
14. Francavilla, C. and C.S. O'Brien (2022) *Open Biol.* **12:**210373.
15. Vogel, A. *et al.* (2023) *Annu. Rev. Med.* **74**:293.
16. Zingg, D. *et al.* (2022) *Nature* **608**:609.

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