Catalog #	Aliquot Size
D30-10G-05	5 µg
D30-10G-10	10 µg

DGKZ, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog # D30-10G
Lot # M2634-10

Product Description

Recombinant full-length human DGKZ was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The DGKZ gene accession number is NM_003646 (Isoform 4).

Gene Aliases

DAGK5; DAGK6; DGK-ZETA; hDGKzeta

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, and 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

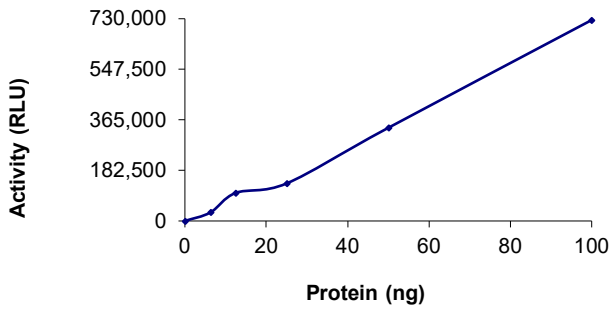
Scientific Background

Diacylglycerol kinase zeta (DAG kinase zeta) is a member of class IV diacylglycerol kinase family, which contains two zinc fingers, an ATP binding site, and four ankyrin repeats with the MARCKS phosphorylation site, and exhibits the preference for 1,2-diacylglycerols but not for long chain diacylglycerols (1). DGK zeta regulates Ras activation via terminating the diacylglycerol signaling induced RasGRP (2). DGK zeta deficient mice were hyper-responsive to T cell receptor stimulation (3). Also, increased expression levels of DGK zeta promotes Rho GTPase activity and cancer cell invasion (4).

References

1. Bunting, M. et al. Molecular Cloning and Characterization of a Novel Human Diacylglycerol Kinase zeta. The Journal of Biological Chemistry. 271:10230–10236, 1996.
2. Topham, M. et al. Diacylglycerol Kinase zeta Regulates Ras Activation by a Novel Mechanism. The Journal of Cell Biology. 152:1135–1143, 2001.
3. Zhong X. et al. Enhanced T cell responses due to diacylglycerol kinase ζ deficiency. Nature Immunology. 4:882-890, 2003.
4. Cai K. et al. Increased diacylglycerol kinase ζ expression in human metastatic colon cancer cells augments Rho GTPase activity and contributes to enhanced invasion. BMC Cancer. 14:208-217, 2014.

Specific Activity



The specific activity of DGKZ was determined to be **18.2 nmol /min/mg** as per activity assay protocol.

Purity

The purity of DGKZ was determined to be **>90%** by densitometry.
Observed approx. MW **150 kDa**; Calculated MW **~132 kDa** (1).

DGKZ, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog #	D30-10G
Specific Activity	18.2 nmol/min/mg
Lot #	M2634-10
Purity	>90%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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ADP-Glo™ Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: D30-10G)

Active DGKZ (0.1μg/μl) diluted with Kinase Dilution Buffer IX (Catalog #: K29-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active DGKZ for optimal results).

Kinase Dilution Buffer IX (Catalog #: K29-09)

Kinase Assay Buffer III (Catalog #: K03-09) diluted at a 1:4 ratio (5X dilution) with cold water. Add freshly 50μM DTT to the aliquot prior to use.

Kinase Assay Buffer III (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM $MgCl_2$ and 0.5 mg/ml BSA.

ADP-Glo™ Kinase Assay Kit (Promega, Cat # V9101)

ATP solution, 10 mM
ADP solution, 10 mM
ADP-Glo™ Reagent
Kinase Detection Reagent

250 μM ATP Assay Solution

Prepare the ATP assay solution by diluting ATP solution, 10 mM (Promega, Cat # V9101) to 250 μM in Kinase Dilution Buffer IX (Catalog #: K29-09).

Substrate (Catalog #: D430-59)

The substrate solution contains 10 mM of Dilauroyl-sn-glycerol in 100% DMSO solution. Dilute the substrate solution to 500 μM in Lipid Dilution Buffer (Catalog #: L21-09) prior to use.

Assay Protocol

The DGKZ assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the DGKZ reaction. The ADP- Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

Step 1. Thaw the Active DGKZ, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.

Step 2. In a pre-cooled 96-well opaque plate, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active DGKZ (Catalog #D30-10G)
- **Component 2.** 5μl of substrate solution
- **Component 3.** 5μl Kinase Dilution Buffer IX with 0.1% Triton X-100

Step 3. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of Kinase Dilution Buffer IX.

Step 4. Initiate the reaction by the addition of 5μl of 250 μM ATP Assay Solution thereby bringing the final volume up to 25μl. Shake the reaction mixture in the 96-well opaque plate for 2 minutes and continue the incubation at 30°C for 40 minutes.

Step 5. After the 40 minute incubation period, terminate the reaction and deplete the remaining ATP by adding 25μl of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minutes at ambient temperature.

Step 6. Then add 50μl of the Kinase Detection Reagent to the 96-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.

Step 7. Read the 96-well reaction plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031).

Step 8. Determine the corrected activity (RLU) by removing the blank control value (see Step 3) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected RLU from reaction / [(SA of ADP in RLU/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)

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SAFETY DATA SHEET

Article 1 - Product Identification

Product Name: DGKZ, Active **Catalog # D30-10G**

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Manufacturer's Name: SignalChem Pharmaceuticals Inc.
Street Address: *110-13120 Vanier Place*
City, Prov. Postal Code: *Richmond, BC, V6V 2J2*
Fax: 604-232-4601
EMERGENCY PHONE: 604-232-4600

Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** Skin irritation (Category 3); Eye irritation (Category 2B).
- **Hazard Pictograms:** none.
- **Signal words:** Warning.
- **Hazard statements:** Causes mild skin irritation (H316); Causes eye irritation (H320).
- **Precautionary statements:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305 + P351 + P338).
- **Other hazards:** none known.

Article 3 – Composition/Information on Ingredients

Chemical Characterization: Mixtures.
Description: This product consists of the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Glycerol	Glycerol	56-81-5	25%
NaCl	Sodium chloride	7647-14-5	0.875%
Tris-HCl; Tris (hydroxymethyl) aminomethane hydrochloride	2 – Amino – 2 - (hydroxymethyl) propane - 1, 3 - diol hydrochloride	1185-53-1	0.788%
Glutathione	Glutathione	70-18-8	0.307%
Protein		No data available	0.01%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.0038%
EDTA	Ethylenediaminetetraacetic acid	6381-92-6	0.0037%
PMSF; Phenylmethanesulfonyl fluoride	a-Toluenesulphonyl fluoride	329-98-6	0.002%

Article 4 – First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breath in fresh air. If cannot breathe, give artificial respiration and consult a physician.
- **After skin contact:** Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- **After eye contact:** Rinse opened eyes with plenty of water for at least 15 minutes. Consult a physician.
- **After swallowing:** rinse the mouth with plenty of water and consult a physician.

Article 5 - Fire-fighting Measures

- **Suitable extinguishing media:** Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- **Specific hazards arising from the substance or mixture:** None known.
- **Special protective equipment and precautions for fire-fighters:** Self-contained breathing apparatus if necessary.

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Article 6 – Accidental Release Measures

- **Personal precautions, protective equipment and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- **Environmental precautions:** Do not allow to enter drains.
- **Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

Article 7 - Handling and Storage

- **Precautions for sate handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store in a dry and well-ventilated place in -70 °C. Keep container upright and tightly closed.

Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:**
  Glycerol (CAS-No: 56-81-5)

Values	Control parameters	Regulations
TWA	10 mg/m³ for mist	British Columbia, Canada
TWA	3 mg/m³ for respirable mist	British Columbia, Canada
TWA	10 mg/m³	Alberta, Canada
TWAEV	10 mg/m³	Ontario, Canada
TWAEV	10 mg/m³	Quebec, Canada
TWA	10 mg/m³	USA

- **Appropriate engineering controls:**
  Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- **Individual protection measures:**
  Respiratory protection:
  Use appropriate respirator if there is inadequate ventilation by following the government standards.
  Hand protection:
  Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.
  Eye/face protection:
  Safety goggles with side-shields approved under appropriate government standards.
  Skin/body protection:
  Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

Article 9 – Physical and Chemical Properties

Appearance: Colorless fluid.	**Danger of explosion:** Product does not present an explosion hazard.
Odour/Odour Threshold: Not determined.	**Explosion limits:** Lower: 0.9 Vol %; Upper: 0.0 Vol %.
pH: Not available.	**Decomposition temperature:** Not available.
Melting point/freezing point: Not determined.	**Vapor pressure at 20 °C:** 0.1 hPa
Boiling point/Boiling range: 100 °C.	**Density:** Not determined.
Flash point: > 100 °C.	**Relative density:** Not determined.
Flammability (solid, gaseous): Not determined.	**Vapor density:** Not determined.
Ignition temperature: 400 °C.	**Evaporation rate:** Not determined.
Auto-igniting: Product is not self-igniting.	**Solubility in / Miscibility with Water:** Fully miscible.

Article 10 - Stability and Reactivity

- **Reactivity:** Stable under recommended transport and storage conditions.
- **Chemical stability:** Stable under recommended transport and storage conditions.
- **Possible hazardous reactions:** No dangerous reactions known.
- **Conditions to avoid:** Heat and moisture.
- **Incompatible materials:** Strong acids/bases, strong oxidizing/reducing agents.
- **Hazardous decomposition products:** Carbon oxides may form under fire conditions; no known decomposition information for other decomposition products.

SAFETY DATA SHEET

Article 11 - Toxicological Information

- **Acute toxicity:** Not available.
- **LD/LC50:** Not available.
- **Skin corrosion/irritation:** Not available.
- **Serious eye damage/eye irritation:** Not available.
- **Respiratory or skin sensitization:** Not available.
- **Germ cell mutagenicity:** Not available.
- **Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- **Reproductive toxicity:** Not available.
- **Teratogenicity:** Not available.
- **Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- **Aspiration hazard:** Not available.
- **Potential health effects:**
  Inhalation: May be harmful if inhaled. May cause respiratory tract irritation.
  Ingestion: May be harmful if swallowed.
  Skin: May be harmful if absorbed through skin. May cause skin irritation.
  Eyes: May cause eye irritation.
- **Signs and Symptoms of Exposure:**
  Prolonged or repeated exposure can cause: Nausea, Dizziness.
- **Synergistic effects:** Not available.

Article 12 - Ecological Information

- **Eco-toxicity:** Not applicable.
- **Biodegradability:** Not applicable.
- **Bio-accumulative potential:** Not applicable.
- **Mobility in soil:** Not applicable.
- **PBT and vPvB assessment:** Not applicable.
- **Other adverse effects:** Not applicable.

Article 13 - Disposal Considerations

- **Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- **Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

Article 14 - Transport Information

- **DOT:** Not dangerous goods.
- **IMDG:** Not dangerous goods.
- **IATA:** Not dangerous goods.

Article 15 – Regulatory Information

- **WHMIS Classification:** Non-hazardous.
- **GHS label elements:** Not applicable.
- **Signal word:** Not applicable.
- **Hazard statements:** Not applicable.

Article 16 - Other Information

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.