Catalog #	Aliquot Size
UK05-E311H-20	20 µg
UK05-E311H-50	50 µg
UK05-E311H-100	100 µg
UK05-E311H-200	200 µg

Uridylate Kinase

Recombinant *E. coli* protein expressed in *E. coli*

Catalog # UK05-E311H

Lot # T4216-9

Product Description

Recombinant full-length *E. coli* Uridylate kinase was expressed in E. coli with a N-terminal His tag. The protein accession number is X78809.

Gene Aliases

pyrH, smbA, UMPK, UMP kinase

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 8.0, 300mM NaCl, 150mM imidazole, 1mM DTT, 10% 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

Uridylate kinase, also known as UMP kinase, in E. coli catalyze the phosphorylation of UMP and ATP to yield UDP and ADP with Mg^{2+} as the cofactor (1). Bacterial UMP kinases are hexamers and are allosterically activated by GTP and allosterically inhibited by UTP (1,2). They are the most studied nucleoside monophosphate (NMP) kinase in their family and have sequence similarity to aspartokinases, glutamate kinases, and Pseudomonas aeruginosa carbamate kinase (2). UMP kinase is a regulatory enzyme in the de novo biosynthetic pathway of pyrimidine molecules (3).

References

1. Briozzo, P. et al. Structure of *Escherichia coli* UMP kinase differs from that of other nucleoside monophospohate kinases and sheds new light on enzyme regulation. J. Biol. Chem. 2005, 280:25533-25540.
2. Serina, L. et al. Escherichia coli UMP-kinase, a member of the aspartokinase family, is a hexamer regulated by guanine nucleosides and UTP. Biochemistry. 1995, 34(15): 5066-5074.
3. Bucurenci, N. et al. Mutational analysis of UMP Kinase from *Escherichia coli*. J Bacteriol. 1998, 180(3): 473-477.

Specific Activity

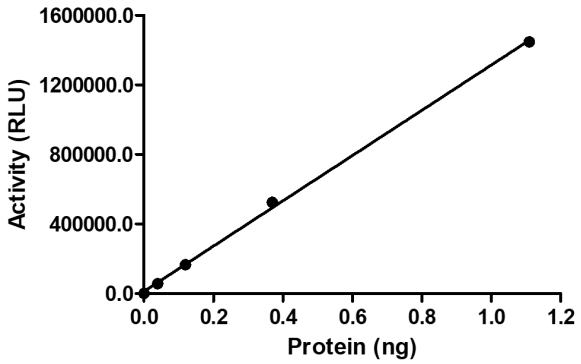

Figure 1. The specific activity of Uridylate Kinase was determined to be **10 µmol/min/mg.**

Purity

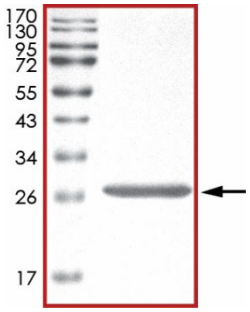

Figure 2. SDS-PAGE gel image

The purity of Uridylate kinase was determined to be **>95%** by densitometry.
Approx. MW **27 kDa.**

Uridylate kinase

Recombinant *E. coli* protein expressed in *E. coli*

Catalog #	UK05-E311H
Specific Activity	10 µmol/min/mg
Lot #	T4216-9
Purity	>95%
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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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: UK05-E311H)

Active Uridylate kinase (0.1µg/µl) 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 Uridylate kinase for optimal results).

Kinase Assay Buffer III (5x) (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM $MgCl_2$ and 0.5mg/ml BSA. Add fresh DTT prior to use to a final concentration of 250µM.

Kinase Dilution Buffer IX (1x) (Catalog #: K29-09)

Kinase Assay Buffer III (Catalog #: K03-09) diluted at a 1:4 ratio (5X dilution) with distilled H_2O. Add fresh DTT prior to use to a final concentration of 50µM.

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

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

10mM Uridine 5'-monophosphate disodium salt (User prepared)

Prepare 10mM stock in distilled water.

0.1M DTT (User prepared)

Prepare 0.1M stock in distilled water.

Assay Protocol

The Uridylate kinase assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the UMP kinase 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 Uridylate kinase, Kinase Assay Buffer, uridine 5'-monophosphate disodium salt, and DTT on ice.

Step 2. In a half area, solid white 96-well plate, add the following reaction components bringing the initial reaction volume up to 20µl:

- **Component 1.** 1ng Active Uridylate kinase (Catalog #: UK05-E311H)
- **Component 2.** ATP solution (to a final concentration of 100µM)
- **Component 3.** Uridine 5'-monophosphate disodium salt (to a final concentration of 100µM)
- **Component 4.** 4µL of 5X Kinase Assay Buffer (K03-09)
- **Component 5.** Distilled water to 20µL

Step 3. Set up the blank control as outlined in step 2, excluding the addition of the Active Uridylate kinase.

Note: A series of ADP standard solutions can be included with the enzyme assay in order to determine the specific activity of the enzyme. Prepare the standard solutions using Kinase Dilution Buffer IX (1x) (Catalog #: K29-09)

Step 4. Shake the reaction mixture in the 96-well plate for 1 minute and incubate the reaction at ambient temperature for 20 minutes.

Step 5. After the 20-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 20µl of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 60 minutes at ambient temperature.

Step 6. Then add 40µl of the Kinase Detection Reagent to the 96-well plate and incubate the reaction mixture for another 60 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. Use an ADP standard curve to determine the concentration of ATP produced (µM) and calculate the kinase specific activity as outlined below.

Kinase Specific Activity (SA) (µmol/min/mg)

$$= \frac{[ADP]\ (\mu M)\ x\ Reaction\ Volume\ (L)}{Reaction\ Time\ (\mathrm{min})\ x\ Enzyme\ Amount\ (mg)}$$

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SAFETY DATA SHEET

Article 1 – Product Identification

Product Name: Uridylate kinase **Catalog # UK05-E311H**

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 Biotech 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	10%
NaCl	Sodium chloride	7647-14-5	1.75%
Imidazole	1,3-Diaza-2,4-cyclopentadiene	288-32-4	≤1.02%
Sodium Phosphate, Dibasic	Sodium Phosphate, Dibasic	7782-85-6	1.34%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.0154%
Protein		No data available	0.01%

Article 4 – First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breathe 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 safe 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 formed under fire conditions; no known decomposition information for other decomposition products.

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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.