Non Radioisotopic Kit for Measuring cGK Activity

CycLex Cyclic GMP dependent protein kinase (cGK) Assay Kit

Cat# CY-1161

Intended Use

The CycLex Research Product **CycLex Cyclic GMP dependent protein kinase (cGK) Assay Kit** is primarily designed to measure the activities of purified the cGK family of kinases for the rapid and sensitive evaluation of inhibitors or activators. The phospho-specific monoclonal antibody used in this assay kit has been demonstrated to recognize the phospho-threonine 68/119 residues on G-kinase substrate, which is phosphorylated by cGK family members. Additionally, column fractions of any cultured primary cell, cell line, or tissue homogenate can be assayed for cGK family activity with the CycLex Research Product **CycLex cGK Assay Kit** if the appropriate dose of cGK specific inhibitor, e.g. (Rp)-8-pCPT-cGMPS.

Applications of this kit include:

1) Monitoring the purification of cGK.
2) Screening inhibitors or activators of cGK.
3) Detecting the effects of pharmacological agents on cGK.

This assay kit is for research use only and not for use in diagnostic or therapeutic procedures.

Storage

- Upon receipt store all components at 4°C.
- Don't expose reagents to excessive light.

Introduction

Nitric oxide (NO) and a broad spectrum of hormones, drugs, and toxins raise intracellular cGMP concentrations and thereby regulate a great variety of functions, including smooth muscle relaxation, neuronal excitability, and epithelial electrolyte transport. Pfeifer *et al.* (1996) noted that depending on the tissue, an increase in cGMP concentration leads to the activation of different receptors, such as cyclic nucleotide phosphodiesterases. The identification of the physiologic mediator of cGMP is complicated by the existence of 2 forms of cGMP-dependent protein kinase (cGK), types I and II, which are encoded by distinct genes. Smooth muscle, platelets, and cerebellum contain high concentrations of cGK-I, whereas cGK-II is highly concentrated in brain, lung, and intestinal mucosa. The function of cGK-II is not well understood, although there is evidence that it mediates intestinal secretion of water and electrolytes induced by the *E. coli* toxin STa and the intestinal peptide guanylin.

Activation of cyclic GMP-dependent protein kinase (cGK) is an important event in the regulation of blood pressure and platelet function. Upstream signals include the generation of nitric oxide (NO) by NO synthases and the subsequent rise in cGMP levels mediated by NO-dependent guanyl cyclases (GCs). The identification of new cGK activators by high throughput screening (HTS) may lead to the development of a novel class of therapeutics for the treatment of cardiovascular diseases.

Measurement of cGK activity

The protocol generally regarded as most sensitive for the quantitative measurement of cGK activity involves incubation of the cGK sample with substrate, either a natural or synthetic polypeptide (such as Histone H1 substrate peptide), in the presence of $Mg^{2+}$and ^{32}P-labeled ATP. The reaction is terminated by "spotting" a sample onto a filter paper disc, followed by immersion in acid to precipitate the radiolabeled product. The filter papers are then washed extensively to remove unincorporated radiolabel and the radioactivity is counted. While sensitive, this method is labor-intensive, generates hazardous radioactive waste, and depends on a radioisotope of short half-life. It is particularly unsuitable when kinase assays are only performed on an infrequent basis. The **CycLex cGK Assay Kit** uses a peroxidase coupled anti-phospho-G-kinase substrate threonine 68/119 monoclonal antibody as a reporter molecule in a 96-well ELISA format. This assay provides a non-isotopic, sensitive and specific method to measure cGK family activity.

Principle of the Assay

The CycLex Research Product **CycLex cGK Assay Kit** is a single-site, semi-quantitative immunoassay for cGK activity. Plates are pre-coated with a substrate corresponding to recombinant G-kinase substrate, which contains threonine residues that can be phosphorylated by cGK family members, including cGKI and cGKII.

The detector antibody specifically reacts with only the phosphorylated form of threonine 68/119 residues on cGK substrate. The **CycLex cGK Assay Kit** may be used to determine the presence of cGK activity in cell lysates, purification column fractions, or to follow the kinetics of a purified or partially purified cGK protein, as well as screening cGK inhibitors. To perform the test, the sample is diluted in Kinase Buffer, pipetted into the wells and allowed to phosphorylate the bound substrate in the presence of Mg^{2+} and ATP. The amount of phosphorylated substrate is measured by binding it with a horseradish peroxidase conjugate of 10H11, a anti-phospho-G-kinase substrate threonine 68/119 specific antibody, which then catalyzes the conversion of the chromogenic substrate tetra-methylbenzidine (TMB) from a colorless solution to a blue solution (or yellow after the addition of stopping reagent). The color is quantified by spectrophotometry and reflects the relative amount of cGK activity in the sample. For kinetic analysis, the sample containing cGK activity is added to the wells in a similar fashion and at varying times the reaction is stopped by the addition of a chelator, sodium ethylenediamine tetraacetate (EDTA) and the amount of phosphorylated substrate determined as before.

The CycLex Research Product **CycLex cGK Assay Kit** is designed to accurately determine the presence and relative amount of cGK activity in tissue cytosols, cell extracts, purification column fractions, and for the non-isotopic kinetic analysis of cGK activity. Careful attention to extraction methods and the assay protocol will provide the investigator with a reliable tool for the evaluation of cGKs.

Summary of Procedure

Add 100 µL of sample to the wells
↓ Incubate for 30 min at 30°C
Wash the wells
↓
Add 100 µL of HRP conjugated anti-phospho-specific antibody
↓ Incubate for 1hr at room temp.
Wash the wells
↓
Add 100 µL of Substrate Reagent
↓
Add 100 µL of Stop Solution
↓
Measure absorbance at 450 nm

Materials Provided

All samples and standards should be assayed in duplicate. The following components are supplied and are sufficient for one 96-well microtiter plate kit.

Microplate: One microplate supplied ready to use, with 96 wells (12 strips of 8-wells) in a foil, zip-lock bag with a desiccant pack. Wells are coated with recombinant G-kinase substrate.

10X Wash Buffer: One bottle containing 100 mL of 10X buffer containing 2 %Tween®-20

Kinase Buffer: One bottle containing 20 mL of 1X buffer; used for Kinase Reaction Buffer and sample dilution.

20X ATP: One vial of lyophilized ATP Na_2 salt.

HRP conjugated Detection Antibody: One vial containing 12 mL of HRP (horseradish peroxidase) conjugated anti-phospho-G-kinase substrate (10H11) antibody. Ready to use.

Substrate Reagent: One bottle containing 20 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use.

Stop Solution: One bottle containing 20 mL of 1 N H_2SO_4. Ready to use.

Materials Required but not Provided

- **cGK positive control-1 (Catalytic domain)**, **cGK positive control-2 (Full length):** Available from CycLex (Cat# CY-E1161-1 and Cat# CY-E1161-2, respectively); One vial contains 4000 units/100 µL cGK enzyme. Positive control should be added to the first well at 2 units/well.
- **cyclic GMP:** guanosine 3':5'-monophosphate is available from Sigma, Cat# G-6129. Make 5 mM stock solution.
- **(Rp)-8-pCPT-cGMPS:** (Rp)-8-(para-chlorophenylthio) guanosine-3':5'-monophosphate is available from Calbiochem, Cat# 370677.
- **10X K252a (10 µM):** K252a is available from Calbiochem, Cat#. 420297. 1 mM stock solution (DMSO) diluted 1:100 in Kinase Buffer.
- **Pipettors:** 2-20 µL, 20-200 µL and 200-1000 µL precision pipettors with disposable tips.
- **Precision repeating pipettor**.
- **Wash bottle or multichannel dispenser** for plate washing.
- **Microcentrifuge and tubes** for sample preparation.
- **Vortex mixer**.
- **Plate reader** capable of measuring absorbance in 96-well plates at dual wavelengths of 450 nm/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. The plate can also be read at a single wavelength of 450 nm, which will give a somewhat higher reading.
- **500 or 1000 mL graduated cylinder**.
- **Reagent reservoirs**.
- **Deionized water of high quality**.

Precautions and Recommendations

- Store the ATP at -20°C in aliquots. Store all other components at 4°C. Do not expose reagents to excessive light. Avoid freeze/thaw cycles.
- Allow all the components to come to room temperature before use.
- All microplate strips that are not immediately required should be returned to the zip-lock pouch, which must be carefully resealed to avoid moisture absorption.
- Do not use kit components beyond the indicated kit expiration date.
- Use only the microtiter wells provided with the kit.
- Rinse all detergent residue from glassware.
- Use deionized water of the highest quality.
- Do not mix reagents from different kits.
- The buffers and reagents in this kit may contain preservatives or other chemicals. Care should be taken to avoid direct contact with these reagents.
- Do not mouth pipet or ingest any of the reagents.
- Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are handled.
- Dispose of tetra-methylbenzidine (TMB) containing solutions in compliance with local regulations.
- Avoid contact with Substrate Solution which contains hydrogen peroxide.
- Avoid contact with Stop Solution which contains Sulfuric Acid.
- In case of contact with the Stop Solution and the Substrate Solution, wash skin thoroughly with water and seek medical attention, when necessary.
- **Biological samples may be contaminated with infectious agents. Do not ingest, expose to open wounds or breathe aerosols. Wear protective gloves and dispose of biological samples properly.**
- **CAUTION: Sulfuric Acid is a strong acid. Wear disposable gloves and eye protection when handling Stop Solution.**

Detailed Protocol

The **CycLex cGK Assay Kit** is provided with removable strips of wells so the assay can be carried out on separate occasions using only the number of strips required for the particular determination. Since experimental conditions may vary, an aliquot of the cGK-I positive control (Cat# CY-E1161), separately available from CycLex, should be included in each assay. Disposable pipette tips and reagent troughs should be used for all transfers to avoid cross-contamination of reagents or samples.

Preparation of Working Solution

1. Prepare a working solution of **Wash Buffer** by adding 100 mL of the **10X Wash Buffer** (provided) to 900 mL of ddH_2O. Mix well. Store at 4°C for two weeks or -20°C for long-term storage.

2. Prepare **20X ATP Solution** by adding **0.8 mL** of ddH_2O to the vial of **20X ATP** (provided, lyophilized). Mix gently until dissolved. The final concentration of the **20X ATP Solution** should be **2.5 mM**. Store the solution in small aliquots (e.g. 100 µL) at -20°C.

3. Prepare **cGMP plus Kinase Reaction Buffer** by mixing following reagents.

	96 assays	**10 assays**
Kinase Buffer (provided)	**9.5 mL**	**950 µL**
20X ATP Solution	**0.5 mL**	**50 µL**
5 mM cGMP	**20 µL**	**2 µL**
Total	**10.02 mL**	**1002 µL**

**You will need 80-90 µL of cGMP plus Kinase Reaction Buffer per assay well. Mix well. Discard any unused cGMP plus Kinase Reaction Buffer after use.*

4. Prepare **Kinase Reaction Buffer** by mixing following reagents. (When you conduct several control experiment described in page 9)

	96 assays	**10 assays**	**1 assay**
Kinase Buffer (provided)	**9.5 mL**	**950 µL**	**95 µL**
20X ATP Solution	**0.5 mL**	**50 µL**	**5 µL**
Total	**10 mL**	**1000 µL**	**100 µL**

Standard Assay

1. Remove the appropriate number of microtiter wells from the foil pouch and place them into the well holder. Return any unused wells to the foil pouch, refold, seal with tape and store at 4°C.

2. Prepare all samples (diluted with Kinase Buffer as needed). All samples should be assayed in duplicate.

3. To assay individual column fractions, add **10 µL** of **each fraction** to the wells of the assay plate on ice. Crude lysates or cell extracts should be added to wells either neat or diluted as described above with Kinase Buffer, if necessary. (Suggested starting dilutions are 1:2, 1:5, and 1:10.) Duplicate wells containing 10 µL of cGK positive control, Cat# CY-E1161, should be included in each assay

as a positive control for phosphorylation.

4. Begin the kinase reaction by addition of **90 µL** of **cGMP plus Kinase Reaction Buffer** per well, cover with plate sealer, and incubate **at 30°C for 30 minutes**.

5. Wash wells five times with Wash Buffer making sure each well is filled completely. Remove residual Wash Buffer by gentle tapping or aspiration.

6. Pipette **100 µL** of **HRP conjugated Detection Antibody** into each well, cover with a plate sealer and incubate **at room temperature (ca.25°C) for 60 minutes**. Discard any unused conjugate.

7. Wash wells five times as same as in step 5.

8. Add **100 µL** of **Substrate Reagent** to each well and incubate **at room temperature (ca.25°C) for 5–15 minutes**.

9. Add **100 µL** of **Stop Solution** to each well in the same order as the previously added Substrate Reagent.

10. Measure absorbance in each well using a spectrophotometric plate reader at dual wavelengths of 450/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. Read the plate at 450 nm if only a single wavelength can be used. Wells must be read within 30 minutes of adding the Stop Solution.

Note-1: Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

Note-2: Reliable signals are obtained when either O.D. values do not exceed 0.25 units for the blank (no enzyme control), or 2.5 units for the cGK positive control.

Note-3: If the microplate reader is not capable of reading absorbance greater than the absorbance of the Wee1 positive control, perform a second reading at 405 nm. A new O.D. values, measured at 405 nm, is used to determine cGK activity of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

Kinetic Assay

1. Remove the appropriate number of microtiter wells from the foil pouch and place them into the well holder. Return any unused wells to the foil pouch, refold, seal with tape and store at 4°C.

2. Prepare all samples (diluted with Kinase Buffer as needed). All samples should be assayed in duplicate.

3. To assay individual column fractions, add **10 µL** of **each fraction** to the wells of the assay plate on ice. Duplicate wells containing 10 µL of cGK positive control, Cat# CY-E1161, should be included in each assay as a positive control for phosphorylation.

4. Begin kinase reaction by addition of **90 µL cGMP plus Kinase Reaction Buffer** in duplicate per well in timed intervals (suggested interval is 5 minutes but should be individually determined for each system). After the final addition, incubate **at 30°C for 20 minutes**.

5. Stop the reaction by flicking out the contents. (Alternatively, the reaction may be terminated by the

addition of 150 µL 0.1 M Na EDTA, pH 8.0 to each well).

6. Wash wells five times with Wash Buffer making sure each well is filled completely. Remove residual Wash Buffer by gentle tapping or aspiration.

7. Pipette **100 µL** of **HRP conjugated Detection Antibody** into each well, cover with a plate sealer and incubate **at room temperature (ca.25°C) for 60 minutes**. Discard any unused conjugate after use.

8. Wash wells same as in Step 6.

9. Add **100 µL** of **Substrate Reagent** to each well and incubate **at room temperature (ca.25°C) for 10-15 minutes**.

10 add **100 µL** of **Stop Solution** to each well in the same order as the previously added Substrate Reagent.

11. Measure absorbance in each well using a spectrophotometric plate reader at dual wavelengths of 450/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. Read the plate at 450 nm if only a single wavelength can be used. Wells must be read within 30 minutes of adding the Stop Solution.

Recommendations

Special considerations when screening activators or inhibitors

In order to estimate the inhibitory effect on cGK family activity in the test chemicals correctly, it is necessary to conduct the control experiment of "Solvent control" at least once for every experiment and "Inhibitor control" at least once for the first experiment, in addition to "Test sample," as indicated in the following table. When test chemicals cause an inhibitory effect on cGK activity, the level of A450 is weakened as compared with "Solvent control". The high level of A450 is not observed in "Inhibitor control" (usually A450<0.2).

Assay reagents	Test sample	Solvent control	Inhibitor control
cGMP plus Kinase Reaction buffer	**80 µL**	**80 µL**	**80 µL**
10X Inhibitor or equivalent	**10 µL**	**-**	**-**
Solvent for Inhibitor	**-**	**10 µL**	**-**
10X K252a (10 µM)*	**-**	**-**	**10 µL**
Cyclex cGK positive control (0.2 units/µL) or your enzyme fraction**	**10 µL**	**10 µL**	**10 µL**

* 10X K252a: See page 4, section "Materials Required but not Provided"
** Cat# CY-E1161-1 and Cat# CY-E1161-2: See page 4, section "Materials Required but not Provided"

1. Following the above table, add the Reagents to each well of the microplate. Finally, initiate reaction by adding 10 µL of "Diluted cGK positive control" (0.2 units/µL) to each well and mixing thoroughly at room temperature. Cover with plate sealer, and incubate **at 30°C for 30 minutes**.

2. Follow the **Standard Assay**, steps 5-10, page 7.

Special considerations when measuring cGK activity

In order to measure the activity of cGK family correctly, it is necessary to conduct the control experiment of "Test Sample cGMP minus" or "Inhibitor control" at least once for every experiment and "ATP minus control" at least once for the first experiment, in addition to "No enzyme control" and "Positive control" as indicated in the following table. Although the level of A450 is several times higher in "Test sample cGMP plus" than in "Test sample cGMP minus" when cGK family enzyme activity is in the sample, the high level of A450 is not observed in "Inhibitor control", "ATP minus control" and "No enzyme control".

Assay reagents	Test Sample cGMP plus	Test Sample cGMP minus	Inhibitor control	ATP minus control	Positive control	No enzyme control
cGMP plus Kinase Reaction buffer	**90 µL**	**-**	**80 µL**	**-**	**90 µL**	**90 µL**
Kinase Reaction buffer	**-**	**90 µL**	**-**	**-**	**-**	**-**
Kinase Buffer (provided)	**-**	**-**	**-**	**90 µL**	**-**	**-**
10X K252a(10 µM)*	**-**	**-**	**10 µL**	**-**	**-**	**-**
Your enzyme fraction	**10 µL**	**10 µL**	**10 µL**	**10 µL**	**-**	**-**
CycLex cGK positive control (0.2 unit/µL)**	**-**	**-**	**-**	**-**	**10 µL**	**-**
Buffer	**-**	**-**	**-**	**-**	**-**	**10 µL**

* 10X K252a: See page 4, section "Materials Required but not Provided"
** Cat# CY-E1161-1 and Cat# CY-E1161-2: See page 4, section "Materials Required but not Provided"

1. Following the above table, add the Reagents to each well of the microplate. Finally, initiate the reaction by adding 10 µL of "Your enzyme fraction" or "Diluted cGK positive control" or "Buffer" to each well and mixing thoroughly at room temperature. Cover with plate sealer, and incubate 30°C for 30 minutes.

2. Follow the **Standard Assay**, steps 5-10, page 7.

Evaluation of Results

1. Average the absorbance values for the cGK sample duplicates (positive control) and all experimental sample duplicate values (when applicable). When the cGK positive control (2 units/assay) is included as an internal control for the phosphorylation reaction, the absorbance value should be greater than 1.0 with a background less than 0.15.

2. For screening of purification/chromatography fractions, plot the mean absorbance values for each of the samples on the Y-axis versus the fraction number on the X-axis to determine the location of the eluted, purified cGK.

3. For kinetic analysis, plot the mean absorbance values for each of the time points on the Y-axis versus the time of each reaction (minutes) on the X-axis.

Assay Characteristics

The CycLex Research Product **CycLex cGK Assay Kit** has been shown to detect the activity of cGK family in column fractions. The assay shows good linearity of sample response. The assay may be used to follow the purification of cGK-containing fractions or may be used to detect the presence of cGK activity in cell lysates.

Troubleshooting

1. The cGK standard should be run in duplicate, using the protocol described in the **Detailed Protocol**. Incubation times or temperatures significantly different from those specified may give erroneous results.

2. The reaction curve is nearly a straight line if the kinetics of the assay are of the first order. Variations in the protocol can lead to non-linearity of the curve, as can assay kinetics that are other than first order. For a non-linear curve, point to point or quadratic curve fit methods should be used.

3. Poor duplicates, accompanied by elevated values for wells containing no sample, indicate insufficient washing. If all instructions in the **Detailed Protocol** were followed accurately, such results indicate a need for washer maintenance.

4. Overall low signal may indicate that desiccation of the plate has occurred between the final wash and addition of Substrate Reagent. Do not allow the plate to dry out. Add Substrate Reagent immediately after wash.

Reagent Stability

All of the reagents included in the CycLex Research Product **CycLex cGK Assay Kit** have been tested for stability. Reagents should not be used beyond the stated expiration date. Upon receipt, kit reagents should be stored at 4°C, except the ATP must be stored at -20°C and cGK positive control (Cat# CY-E1161) must be stored at below -70°C. Coated assay plates should be stored in the original foil bag sealed by the zip lock and containing a desiccant pack.

For research use only, not for use in diagnostic or therapeutic procedures

Sample Preparation

Numerous extraction and purification methods can be used to isolate cGKs. The following protocols have been shown to work with a number of different tissues and enzyme sources and are provided as examples of suitable methods. Crude samples can frequently be used without dilution while more concentrated or highly purified cGKs should be diluted. It is strongly advised that the user always perform an initial experiment to determine the proper dilution to be used in subsequent experiments. This need not be any more than a single time point assay using serial dilutions of the crude extract, cell lysate or sample fraction taken prior to a purification step. One eight well strip of the substrate plate should be sufficient for this initial experiment. All sample preparations should be performed at 4°C and recovered fractions be kept at 4°C to prevent loss of enzymatic activity.

CAUSION: It should be noted that this assay kit detects not only cGK activity but also other protein kinases in crude extract and column sample. You should trace cGK protein level by western blotting in column fractions.

Column Purification Fractions

1. Homogenize fresh 10-15 g of tissues (lung, cerebellum, etc.) in three volumes of extraction buffer (50 mM potassium phosphate buffer, pH 7.0, 1 mM EDTA, 1 mM EGTA, 0.2 mM PMSF, 1 µg/mL pepstatin, 0.5 µg/mL leupeptin, 5 mM beta-glycerophosphate, 2 mM NaF, 2 mM Na_3VO_4, 10 mM beta-mercaptoethanol) in a Potter-Elvehjem tissue homogenizer.

2. Centrifuge the homogenate for 20 min. at 30,000 x g to pellet the insoluble membrane/organelle fraction.

3. Dilute clear lysates by adding 4 volumes of ice-cold 5 mM potassium phosphate buffer, pH 7.0, 10 mM beta-mercaptoethanol.

4. Adjust the pH of the lysates solution to 5.4 by slow addition of 0.5 N acetic acid with stirring.

5. After stirring for 10 min, centrifuge the solution for 15 min. at 30,000 x g to obtain the precipitate, which contains most of cGK enzyme activity.

6. Dissolve the precipitate in 5 ml of DE-buffer (20 mM Tris-HCl, pH 7.5, 60 mM NaCl, 0.5 mM EDTA, 1 mM EGTA, 0.2 mM PMSF, 1 µg/mL pepstatin, 0.5 µg/mL leupeptin, 2 mM NaF, 0.2 mM Na_3VO_4, 10 mM beta-mercaptoethanol).

7. Remove the precipitate by centrifuge the solution for 15 min. at 30,000 x g

8. Apply the supernatant to a 1 x 8 cm column of DEAE cellulose (Whatman DE-52) equilibrated with DE-buffer.

9. Wash the column with five column volumes of DE-buffer.

10. Elute the column with a linear gradient of 0.06-0.3 M NaCl (total six column volumes) in DE-buffer collecting 1-2 mL fractions. These samples are now ready for analysis according to the instructions provided in the **Detailed Protocol**.

Cell Culture Lysates

1. Harvest and pellet cells by centrifugation using standard methods.

2. Resuspend the cell pellet with an appropriate extraction buffer (for example; 20 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.2 mM PMSF, 1 µg/mL pepstatin, 0.5 µg/mL leupeptin, 2 mM NaF, 0.2 mM Na3VO4, 5 mM beta-mercaptoethanol) and lyse the resuspended cells using either a Dounce Homogenizer, sonication, or three cycles of freezing and thawing.

3. Transfer extracts to microcentrifuge tubes and centrifuge for 5 minutes.

4. Aliquot cleared lysate to a clean microfuge tube. These samples are now ready for analysis according to the instructions provided in the **Detailed Protocol**.

NOTE: THE ABOVE PROCEDURES ARE INTENDED ONLY AS A GUIDELINE. THE OPTIMAL EXPERIMENTAL CONDITIONS WILL VARY DEPENDING ON THE PARAMETERS BEING INVESTIGATED, AND MUST BE DETERMINED BY THE INDIVIDUAL USER. NO WARRANTY OR GUARANTEE OF PERFORMANCE USING THESE PROCEDURES IS MADE OR IMPLIED.

Example of Test Results

Fig.1 Dose dependency of cGK

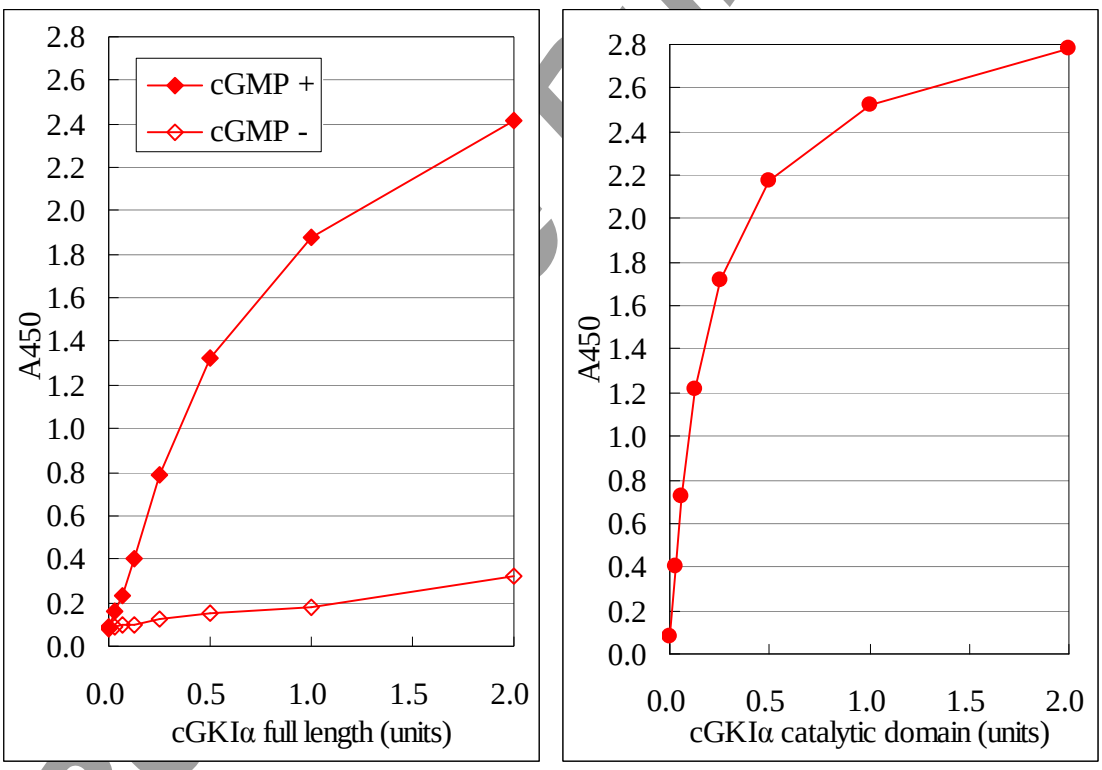


Fig.2 Time course of cGK kinase reaction

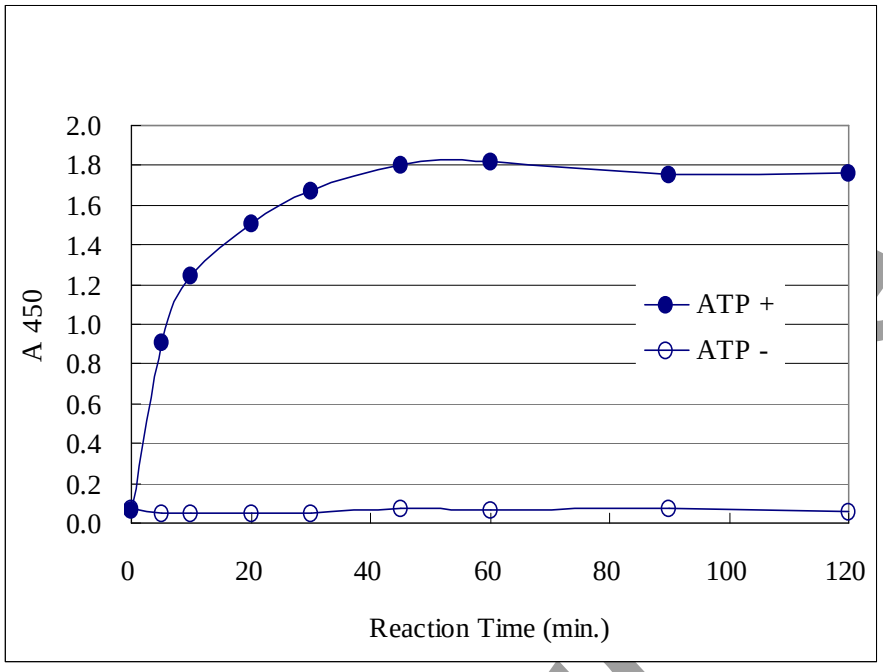


Fig.3 Km for ATP

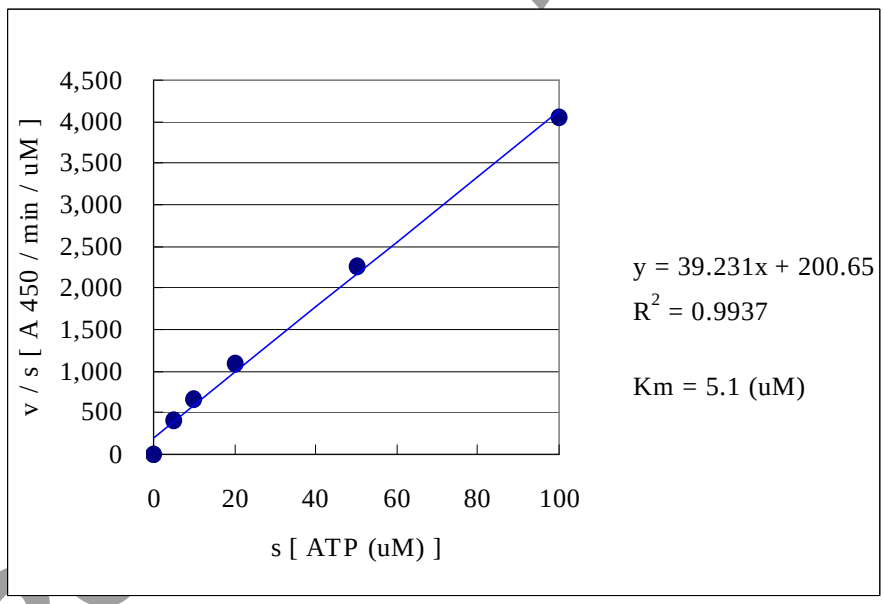


Fig.4 Effect of broad-spectrum kinase inhibitor K252a on cGK I alpha activity

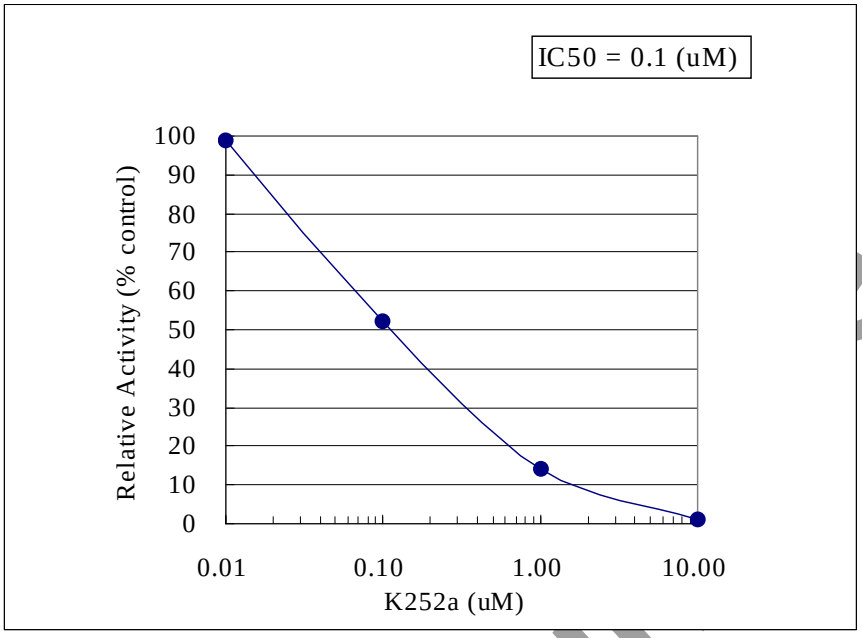


Fig.5 Activation of full length cGK I alpha expressed in 293T cell by cGMP *in Vitro*

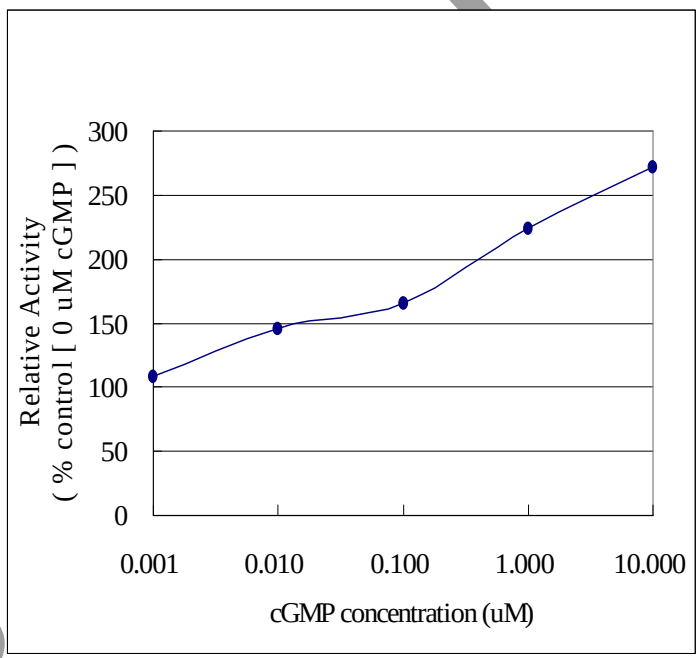


Fig.6 Activation of full length cGK I alpha expressed in 293T cell by 8-CPT-cGMP and SNP-1

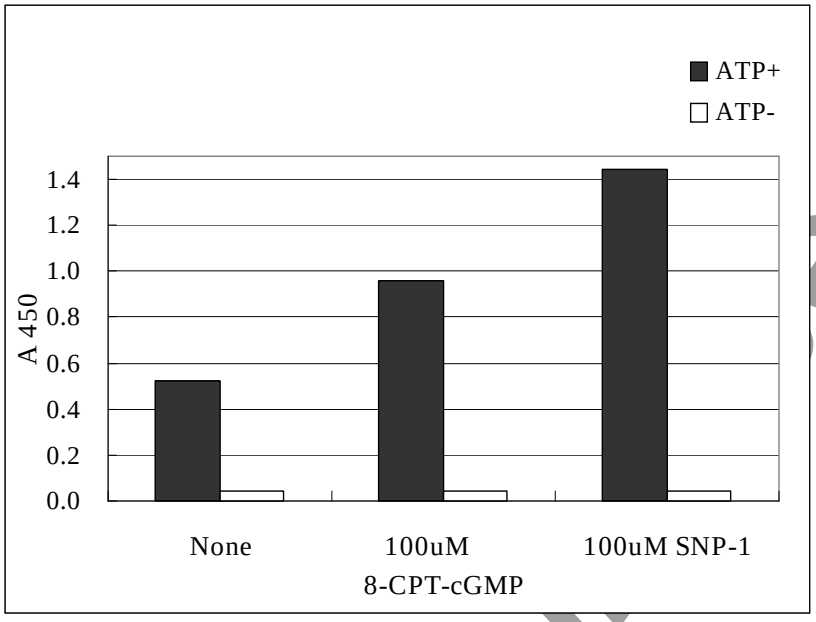


Fig.7 Inhibition of cGK activity that is stimulated with 1 µM cGMP by Rp-8-CPT-cGMP *in vitro*

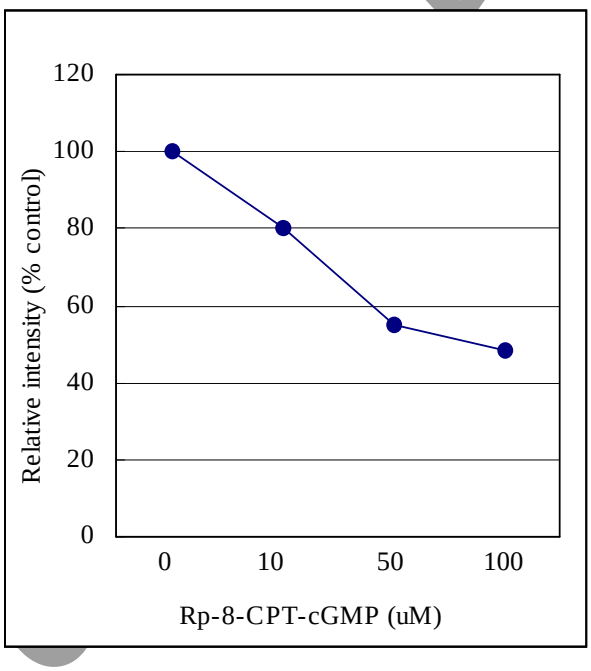


Fig.8 RESOURCE Q column elution profile of cGK (cGK enriched fraction concentrated by isoelectric-point precipitation from rabbit brain extract)

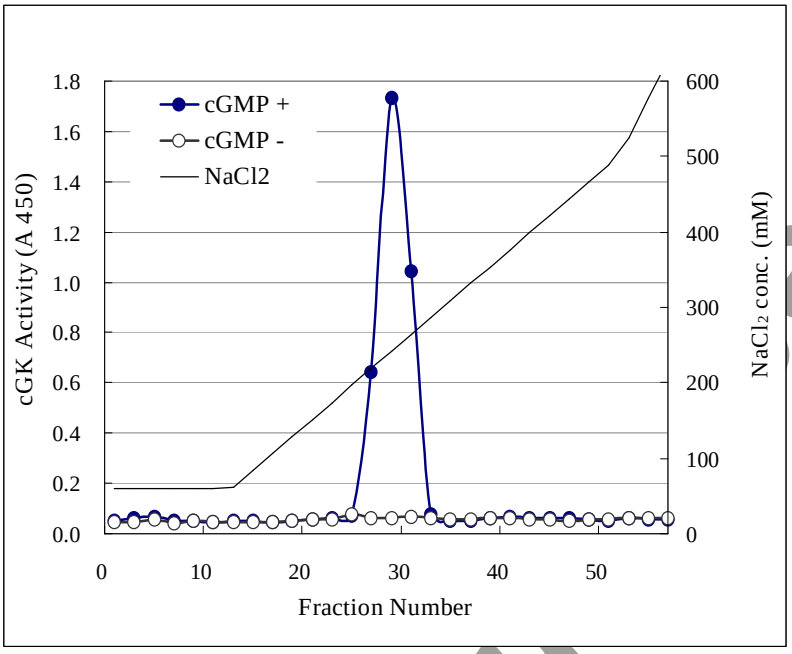
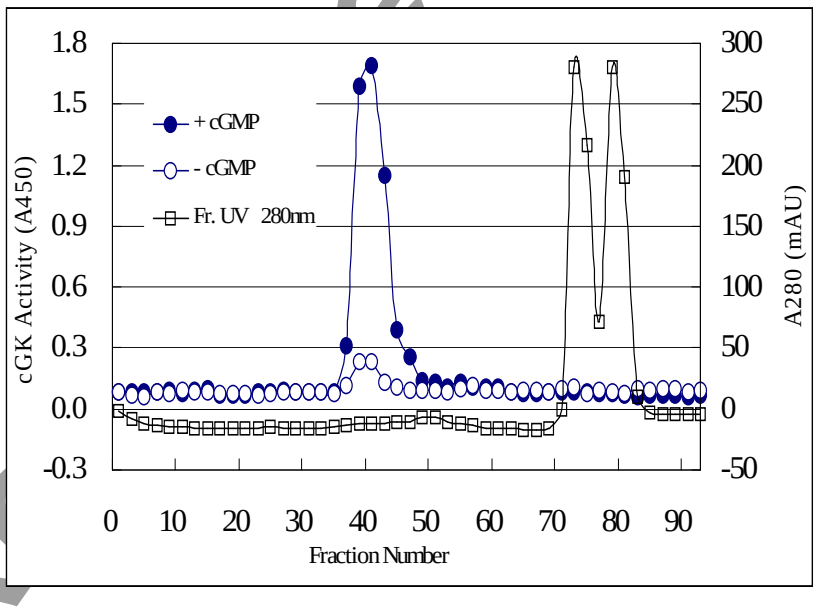


Fig.9 Superdex-200 elution profile of cGK (cGK enriched fraction separated by RESOURCE Q column (Fr.27-32))



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