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ab139437 PKC Kinase Activity Assay Kit

For the rapid, sensitive and accurate measurement of PKC in various samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

PKC Kinase Activity Kit (ab139437) provides a simple non-radioactive and reliable method for quantitating the activity of all isoforms of PKC in crude or partially purified lysates in less than 4.5 hours. It can also be used for screening pharmacological inhibitors or activators of PKC.

In this assay, a synthetic peptide is used as PKC specific substrate. The substrate, which has pre-coated on the wells on the microplate well provided, is phosphorylated by the PKC present in the sample after addition of ATP. The phosphorylated substrate is recognized by a polyclonal antibody that recognizes only the phospho-substrate. The phospho-specific antibody is subsequently recognized a HRP-conjugated secondary antibody. The assay is finally developed with TMB substrate and color develops in proportion to the PKC activity existing in the sample. The color development is stopped with acid stop solution and the intensity of the color is measured in a microplate reader at OD 450 nm.

Protein Kinase C (PKC, EC 2.7.11.13) is a large superfamily of serine/threonine kinases that mediate essential cellular signals required for activation, proliferation, differentiation and survival. Because of its key role in multiple cellular functions, PKC has become an important drug target.

There are at least fifteen PKC isoforms that share similar structure but have distinct distribution patterns and function. The PKC isoforms can be subdivided into three classes based on primary structure and biochemical properties. These are: classical or conventional PKC isoforms (cPKC), novel PKC isoforms (nPKC) and atypical PKC isoforms (aPKC). All PKC isoforms share a characteristic sequence motif C1 in addition to a serine/threonine-protein kinase domain. Studies indicate that the isotype-specific physiological function of PKC is regulated by three events: maturation, catalytic activation and targeting.

2. Protocol Summary

Positive control (active PKC) preparation



Sample preparation



Prepare wells with Kinase Dilution buffer
and add samples



Add ATP and initiate kinase reaction.
Incubate for 90 minutes at 30°C



Add phospho-specific antibody to each well.
Incubate for 60 minutes at RT.



Add HRP-conjugated secondary antibody.
Incubate for 30 minutes at RT.



Wash wells.
Add TMB solution and incubate 30 – 60 minutes at RT.



Add Stop Solution.
Measure optimal density (OD450 nm)

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C in the dark (store active Kinase at -80°C) immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition (Before prep)	Storage Condition (After prep)
20X Wash Buffer	30 mL	4°C	4°C
Active PKC	30 µL	-80°C	-80°C
Antibody Dilution Buffer	10 mL	4°C	4°C
Anti-Rabbit IgG-HRP Conjugate	20 µL	4°C	4°C
ATP	2 mg	4°C	-20°C
Kinase Assay Dilution Buffer	10 mL	4°C	4°C
PKC phosphospecific Substrate Antibody	5 mL	4°C	4°C
PKC Substrate Microtiter Plate	1 unit	4°C	4°C
Stop Solution 2	10 mL	4°C	4°C
TMB Substrate	10 mL	4°C	4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 450 nm
- MilliQ water or other type of double distilled water (ddH₂O)
- PBS
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- Adhesive plate sealers
- Adsorbent paper for blotting
- Lysis Buffer for cell or tissue lysate preparation [20 mM MOPS, 50 mM β -glycerolphosphate, 50 mM sodium fluoride, 1 mM sodium orthovanadate, 5 mM EGTA, 2 mM EDTA, 1% NP40, 1 mM DTT, 1 mM benzamidine, 1 mM PMSF, 10 μ g/mL leupeptin and aprotinin]
- Cell scraper (if using adherent cells)
- Dounce homogenizer (if using tissue)
- BCA protein determination method: we recommend BCA protein assay kit reducing agent compatible (test tube) (ab207004)

For partially purifying cell/tissue extracts:

- Mono Q anion exchange column
- Buffer A [10 mM MOPS pH7.2, 25 mM β -glycerolphosphate, 5 mM EGTA, 2 mM ETA, 2 mM sodium orthovanadate and 2 mM DTT]
- 0.8 M NaCl solution

8. Technical Hints

- This kit is sold based on number of tests. A “test” simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- When aspirating, tilt plate slightly to carefully remove liquid from the well.
- Consistent thorough washing of each well is critical. If using an automatic washer, check washing head before use. If washing manually, ensure all wells are completely filled at each wash. Air bubbles should be avoided.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 20X Wash Buffer:

Equilibrate 20X Wash Buffer to room temperature and swirl gently to dissolve any crystals that may have formed during storage. Prepare **1X Wash Buffer** by diluting 20X Wash Buffer in 570 mL of ddH₂O (total volume 1X Wash Buffer: 600 mL). Store 1X Wash Buffer at room temperature for up to 4 weeks or at 4°C for longer term storage.

9.2 Active PKC (positive control):

Provided at 20 ng/μL. Please refer to vial for the lot specific concentration (ng/μL) of the purified kinase preparation. Thaw kinase on ice and keep on ice while in use. Aliquot active PKC so that you have enough volume to perform the desired number of assays. Store at -80°C and subsequently thaw aliquot only once. Refrozen aliquots may result in a reduction in kinase activity.

Immediately prior to use, make serial dilutions (1:2 sequential dilutions) of active PKC in Kinase Assay Dilution Buffer to a final volume of 30 μL.

9.3 Antibody Dilution Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

9.4 Anti-Rabbit IgG-HRP Conjugate:

Prepare Conjugate Working Solution by diluting conjugate 1:1000 to a final concentration of 1 μg/mL with Antibody Dilution Buffer. Mix gently by inversion.

For 96 wells, a minimum of 4 mL of Conjugate Working Solution is needed. If you are only using a portion of the plate, dilute only what is needed for the number of wells used. Store any remaining undiluted conjugate at 4°C. Discard any remaining diluted Conjugate Working Solution.

9.5 ATP:

Reconstitute ATP in 2 mL of Kinase Assay Dilution Buffer. Mix gently by inversion. Keep on ice while in use. Aliquot diluted ATP so that you have enough volume to perform the desired number of assays. Store at -20°C for up to 6 months.

9.6 Kinase Assay Dilution Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

9.7 PKC Phosphospecific Substrate Antibody:

Ready to use as supplied. Keep on ice while in use. Store at 4°C.

9.8 PKC Substrate Microtiter Plate:

Ready to use as supplied. The wells should be used in the frame provided. Equilibrate to room temperature before use. Store unused strips in the foil bag with the desiccant at 4°C.

9.9 Stop Solution 2:

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

9.10 TMB Substrate:

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

10. Sample Preparation

General sample information:

- We recommend performing serial dilutions of your sample to ensure linear readings.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C . When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step.
- Add protease inhibitors to Lysis Buffer (see Materials Required Not Provided section) immediately prior use.

CRUDE SAMPLE PREPARATION

Crude sample preparation can be used with the assay. However, these samples may contain other kinases that can phosphorylate the substrate.

10.1 Adherent cell samples:

- 10.1.1 Grow cells to desired plate confluence. Initial recommendation: 90% confluency/100 mm dish.
- 10.1.2 Treat cells with activator/inhibitor as desired. Have an untreated sample as control.
- 10.1.3 Remove culture media from plate.
- 10.1.4 Add 1 mL of Lysis Buffer to 100 mm tissue culture plate and allow to stand on ice for 10 minutes.

Δ Note: Increase/decrease volume of Lysis Buffer accordingly for bigger/smaller tissue culture plates.

- 10.1.5 Scrape cells with a cell scraper and collect lysate in a pre-chilled microcentrifuge tube.
Alternatively, sonicate lysate with 3 x 20 second intervals.
- 10.1.6 Centrifuge lysate at 13,000 rpm for 15 minutes.
- 10.1.7 Transfer clear supernatant to a new pre-chilled microcentrifuge tube. Keep on ice.

Δ Note: Prepared samples can be stored at -80°C . Be aware however that kinase activity decreases with each subsequent freeze/thaw cycle.

- 10.1.8 Determine protein concentration using BCA method.

10.2 Suspension cell samples:

- 10.2.1 Grow cells and treat with activator/inhibitor as desired. Have an untreated sample as control.
- 10.2.2 Harvest cells in a 15 mL conical tube. Initial recommendation: $0.5 - 1 \times 10^7$ cells.
- 10.2.3 Centrifuge cells at 1,200 rpm for 5 – 10 minutes. Optional: wash cells in 5 mL of ice-cold PBS.
- 10.2.4 Resuspend cells in 1 mL of Lysis Buffer by pipetting up and down and incubate tube on ice for 10 minutes. Alternatively, sonicate lysate with 3 x 20 second interval.
- 10.2.5 Centrifuge lysate at 13,000 rpm for 15 minutes.
- 10.2.6 Transfer clear supernatant to a new pre-chilled microcentrifuge tube. Keep on ice.

Δ Note: Prepared samples can be stored at -80°C. Be aware however that kinase activity decreases with each subsequent freeze/thaw cycle.

- 10.2.7 Determine protein concentration using BCA method.

10.3 Tissue samples:

- 10.3.1 Harvest 1 g of tissue (initial recommendation).
- 10.3.2 Wash in cold PBS.
- 10.3.3 Resuspend tissue in 5 mL of Lysis Buffer.
- 10.3.4 Homogenize tissue with a Dounce homogenizer or pestle sitting on ice, with 10 – 15 passes.
- 10.3.5 Allow lysate to stand on ice for 10 minutes.
- 10.3.6 Transfer tissue lysate to a pre-chilled 15 mL conical tube and centrifuge at 13,000 – 14,000 rpm for 30 minutes at 4°C.
- 10.3.7 Transfer clear supernatant to a pre-chilled microcentrifuge tube. Keep on ice.

Δ Note: Prepared samples can be stored at -80°C. Be aware however that kinase activity decreases with each subsequent freeze/thaw cycle.

- 10.3.8 Determine protein concentration using BCA method.

PARTIALLY PURIFIED SAMPLE PREPARATION

Partial purification of cell or tissue extracts is recommended to ensure kinase activity detected is specific for PKC.

10.4 Sample Mono Q Anion Exchange Protocol:

- 10.4.1 Prepare cell or tissue extracts as outlined in previous sections.
- 10.4.2 Equilibrate a 1 mL Mono Q anion exchange column with Buffer A [10 mM MPOS pH7.2, 25 mM β -glycerolphosphate, 5 mM EGTA, 2 mM ETA, 2 mM sodium orthovanadate and 2 mM DTT]
- 10.4.3 Load cell/tissue extracts (1 – 2 mg of protein) onto the Mono Q anion exchange column and run at a flow-rate of 0.5 mL/min using a 12 mL linear NaCl gradient (0 – 0.8 M NaCl).
- 10.4.4 Collect between 0.25 – 0.5 mL fractions.
- 10.4.5 Assay fractions as outlined in Section 11.

11. Assay Procedure – PKC Kinase activity

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.
- Prepare all reagents and samples as directed in the previous sections.
- For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 15 minutes.

Δ Note: Before performing the kinase assay, we strongly recommend that you perform an initial experiment to determine the appropriate dilution of the (purified) sample and reaction time to carry out subsequent studies.

- Perform a time course using various kinase concentrations, including a no-enzyme blank, to confirm a linear response of the kinase with respect to phosphorylation.
- Select a reaction time and kinase concentration from the results obtained in the previous step. This will provide a sufficient window of phosphorylation to yield statistically reliable results.

11.1 Preparation of PKC substrate plate:

- 11.1.1 Determine the number of wells to be used. If less than 96 wells are needed, return unused wells to foil pouch.
- 11.1.2 Soak each well of the PKC substrate microtiter plate with 50 μ L of Kinase Assay Dilution Buffer (step 9.6) at room temperature for 10 minutes.
- 11.1.3 Carefully aspirate liquid from all wells.

11.2 Assay wells set up:

Δ Note: Set up serial dilutions of positive control and samples (crude or purified extracts) to ensure linear reading. Dilute sample in Kinase Assay Dilution Buffer.

- Positive control: serial dilutions of purified active PKC (step 9.2) = 30 μ L active PKC dilutions.
- Sample wells = 30 μ L serial dilutions of samples.
- Blank = 30 μ L Kinase Assay Dilution Buffer.

11.3 Kinase reaction:

- 11.3.1 Initiate reaction by adding 10 µL of reconstituted ATP (step 9.5) to each well except the blank.
- 11.3.2 Cover wells with an adhesive plate sealer or plastic wrap and incubate at 30°C for up to 90 minutes, preferably with gentle, thorough shaking every 20 minutes by hand or a shaker with rotate angle at 60 rpm. Thorough mixing is recommended to yield optimal result.

Δ Note: Use predetermined time point generated during the initial experimental optimization set up.

- 11.3.3 Stop reaction by emptying contents of each well. Invert the plate and carefully pat dry on clean paper towels.

11.4 Phosphospecific substrate antibody incubation:

- 11.4.1 Add 40 µL of the PKC phosphospecific substrate antibody (step 9.8) to each well except the blank.
- 11.4.2 Cover wells with a fresh adhesive plate sealer (or plastic wrap) and incubate at room temperature for 60 minutes, preferably with gentle, thorough shaking every 20 minutes.

11.5 Washing step:

- 11.5.1 Aspirate liquid from all wells.
- 11.5.2 Add 100 µL of 1X Wash Buffer (Step 9.1) to all wells.
- 11.5.3 Repeat washing step three more times with 1X Wash Buffer (total of 4 washes).

Δ Note: To reduce background, it may be necessary to wait 1 – 2 minutes between each wash

- 11.5.4 After the 4th wash, aspirate the liquid from all wells. Invert the plate and carefully pat dry on clean paper towels.

11.6 Anti-rabbit IgG-HRP conjugate incubation:

- 11.6.1 Add 40 µL of diluted anti-rabbit IgG-HRP conjugate (step 9.4) to each well except the blank.
- 11.6.2 Cover wells with a fresh adhesive plate sealer (or plastic wrap) and incubate at room temperature for 30 minutes, preferably with gentle, thorough shaking every 10 minutes.

11.7 Washing step:

- 11.7.1 Aspirate liquid from all wells.
- 11.7.2 Add 100 µL of 1X Wash Buffer (Step 9.1) to all wells.
- 11.7.3 Repeat washing step three more times with 1X Wash Buffer (total of 4 washes).

Δ Note: To reduce background, it may be necessary to wait 1 – 2 minutes between each wash

11.7.4 After the 4th wash, aspirate the liquid from all wells. Invert the plate and carefully pat dry on clean paper towels.

11.8 Detection step:

11.8.1 Add 60 µL of TMB substrate to each well.

11.8.2 Incubate plate at room temperature for 30 – 60 minutes. Incubation time should be monitored by researcher according to color development.

11.8.3 Add 20 µL of Stop Solution 2 to each well, in the same order that the TMB substrate was added.

11.9 Plate reading:

11.9.1 Measure absorbance increase on a microplate reader at OD = 450 nm.

12. Assay Procedure – Inhibitor or Activator Screening

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.
- Prepare all reagent and samples as directed in the previous sections.
- For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 15 minutes.

Δ Note: Before performing the kinase assay, we strongly recommend that you perform an initial experiment to determine the appropriate dilution of the (purified) sample and reaction time to carry out subsequent studies.

- Perform a time course using various kinase concentrations, including a no-enzyme blank, to confirm a linear response of the kinase with respect to phosphorylation.
- Select a reaction time and kinase concentration from the results obtained in the previous step. This will provide a sufficient window of phosphorylation to yield statistically reliable results.

12.1 Sample preparation:

- 12.1.1 Dilute activator or inhibitor in the appropriate solvent.
- 12.1.2 Incubate purified PKC in the presence of activator or inhibitor. Reaction time should be pre-determined by the researcher during the initial experimental optimization set up.

12.2 Preparation of PKC substrate plate:

- 12.2.1 Determine the number of wells to be used. If less than 96 wells are needed, return unused wells to foil pouch.
- 12.2.2 Soak each well of the PKC substrate microtiter plate with 50 μ L of Kinase Assay Dilution Buffer (step 9.6) at room temperature for 10 minutes.
- 12.2.3 Carefully aspirate liquid from all wells.

12.3 Assay wells set up:

- Positive control: serial dilutions of purified active PKC (step 9.2) = 30 μ L active PKC dilutions.
- Sample wells = 30 μ L active PKC incubated with activator or inhibitor.
- Negative control = 30 μ L active PKC incubated with solvent.
- Blank = 30 μ L Kinase Assay Dilution Buffer.

12.4 Kinase reaction:

- 12.4.1 Initiate reaction by adding 10 μ L of reconstituted ATP (step 9.5) to each well except the blank.
- 12.4.2 Cover wells with an adhesive plate sealer or plastic wrap and incubate at 30°C for up to 90 minutes, preferably with gentle, thorough shaking every 20 minutes by hand or a shaker with rotate angle at 60 rpm. Thorough mixing is recommended to yield optimal result.

Δ Note: Use predetermined time point generated during the initial experimental optimization set up.

- 12.4.3 Stop reaction by emptying contents of each well. Invert the plate and carefully pat dry on clean paper towels.

12.5 Phosphospecific substrate antibody incubation:

- 12.5.1 Add 40 μ L of the PKC phosphospecific substrate antibody (step 9.8) to each well except the blank.
- 12.5.2 Cover wells with a fresh adhesive plate sealer (or plastic wrap) and incubate at room temperature for 60 minutes, preferably with gentle, thorough shaking every 20 minutes.

12.6 Washing step:

- 12.6.1 Aspirate liquid from all wells.
- 12.6.2 Add 100 μ L of 1X Wash Buffer (Step 9.1) to all wells.
- 12.6.3 Repeat washing step three more times with 1X Wash Buffer (total of 4 washes).

Δ Note: To reduce background, it may be necessary to wait 1 – 2 minutes between each wash

- 12.6.4 After the 4th wash, aspirate the liquid from all wells. Invert the plate and carefully pat dry on clean paper towels.

12.7 Anti-rabbit IgG-HRP conjugate incubation:

- 12.7.1 Add 40 μ L of diluted anti-rabbit IgG-HRP conjugate (step 9.4) to each well except the blank.

- 12.7.2 Cover wells with a fresh adhesive plate sealer (or plastic wrap) and incubate at room temperature for 30 minutes, preferably with gentle, thorough shaking every 10 minutes.

12.8 Washing step:

- 12.8.1 Aspirate liquid from all wells.
12.8.2 Add 100 μ L of 1X Wash Buffer (Step 9.1) to all wells.
12.8.3 Repeat washing step three more times with 1X Wash Buffer (total of 4 washes).

Δ Note: To reduce background, it may be necessary to wait 1 – 2 minutes between each wash

- 12.8.4 After the 4th wash, aspirate the liquid from all wells. Invert the plate and carefully pat dry on clean paper towels.

12.9 Detection step:

- 12.9.1 Add 60 μ L of TMB substrate to each well.
12.9.2 Incubate plate at room temperature for 30 – 60 minutes. Incubation time should be monitored by researcher according to color development.
12.9.3 Add 20 μ L of Stop Solution 2 to each well, in the same order that the TMB substrate was added.

12.10 Plate reading:

- 12.10.1 Measure absorbance increase on a microplate reader at OD = 450 nm.

13. Calculations

PKC KINASE ACTIVITY IN CELL LYSATES

- Subtract blank reading from samples if significant.
- Average the duplicate reading for each sample.
- Calculate relative kinase activity with the following equation:

Relative Kinase Activity =

$$\frac{\text{Avg absorbance (sample)} - \text{Avg absorbance (blank)}}{\text{Amount of crude protein used per assay}} \times \text{Dilution factor}$$

PKC KINASE ACTIVITY IN COLUMN FRACTIONS

- Subtract blank reading from samples if significant.
- Average the duplicate reading for each sample.
- Calculate relative kinase activity with the following equation:

Relative Kinase Activity =

$$\frac{\text{Avg absorbance (sample)} - \text{Avg absorbance (blank)}}{\text{Volume used in assay}}$$

PKC KINASE ACTIVITY FROM PURIFIED KINASE

- Subtract blank reading from samples if significant.
- Average the duplicate reading for each sample.
- Calculate relative kinase activity with the following equation:

Relative Kinase Activity =

$$\frac{\text{Avg absorbance (sample)} - \text{Avg absorbance (blank)}}{\text{Amount of purified kinase used per assay}}$$

14. Typical Data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

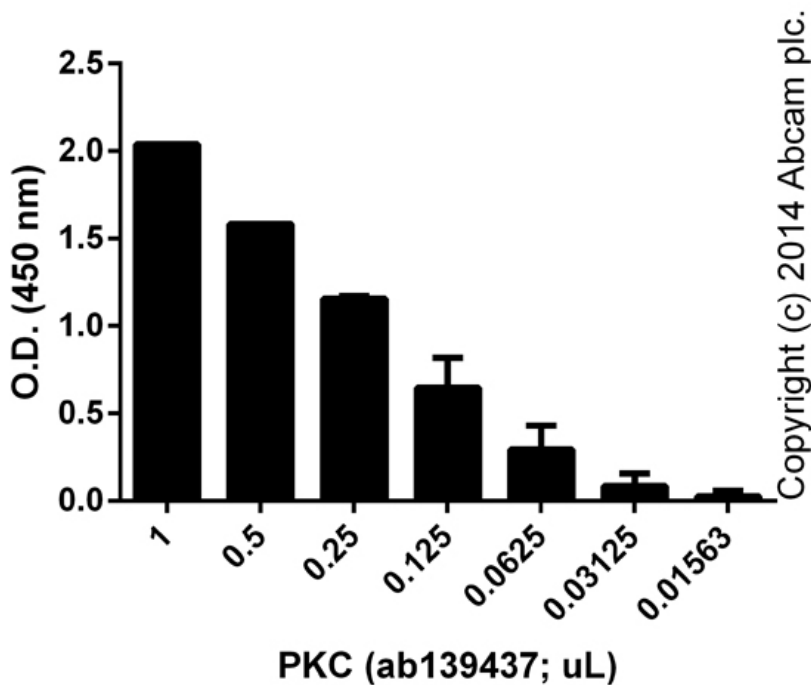


Figure 1. Titration of positive control included in the kit (active PKC) (duplicates; \pm SD). Varying quantities of purified active PKC were assayed using the PKC Substrate Microtiter Plate. Assays were incubated for 60 min. at 30°C.

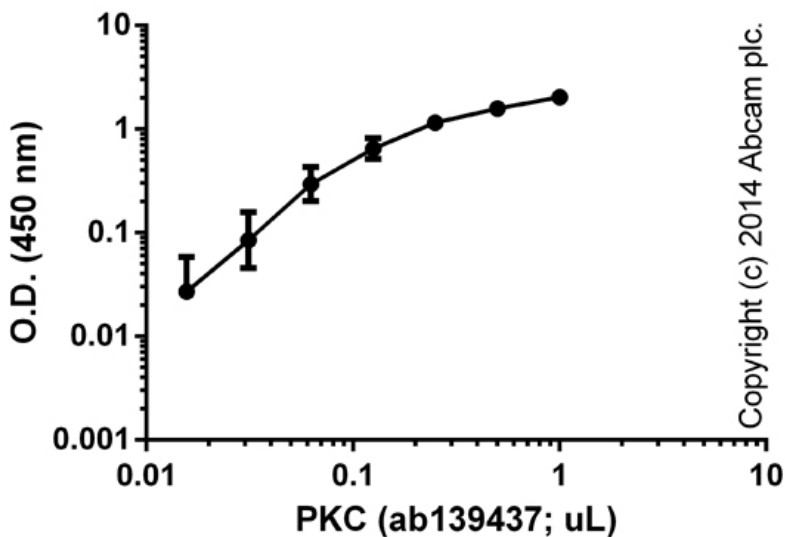


Figure 2. Titration of positive control included in the kit (active PKC). Background signal subtracted (duplicates; \pm SD). Varying quantities of purified active PKC were assayed using the PKC Substrate Microtiter Plate. Assays were incubated for 60 min. at 30°C.

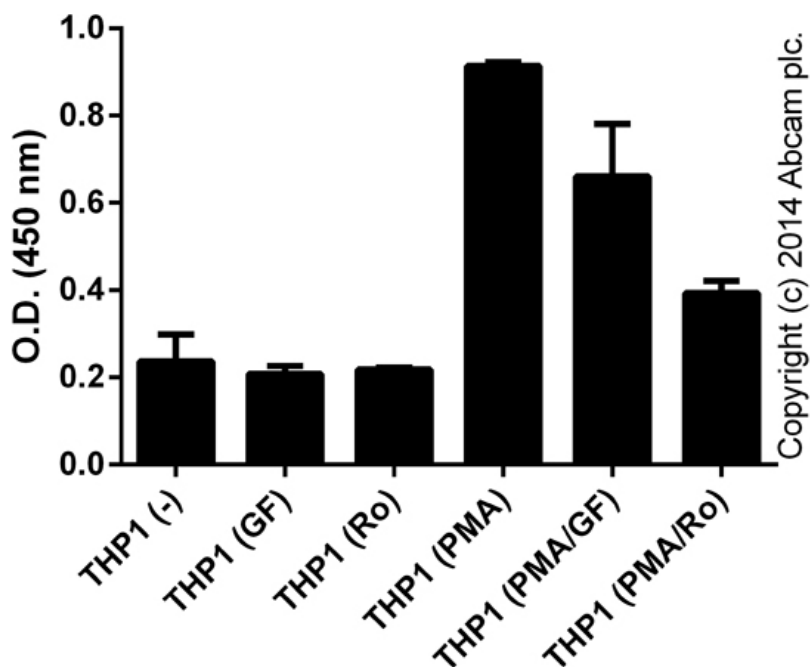


Figure 3. THP-1 cells (1.5×10^7 cells/assay) were treated with 100 nM GF109203X (GF, ab144264) or Ro31-8220 mesylate (Ro, ab120374) for 30 minutes prior to activation with 10 μ g/mL PMA (Sigma) for 4 hours. Controls cells were left without inhibitors or PMA. Cells were lysed in 1 mL of lysis buffer, and 30 μ L were tested for PKC activity (duplicates; \pm SD).

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents; get equipment ready.
- Prepare samples (untreated/treated) in optimal dilutions to fit linear range readings.
- Soak wells of the PKC substrate microtiter plate with 50 μ L Kinase Assay Dilution Buffer at RT for 10 min. Carefully aspirate liquid from each well.
- Add blank (30 μ L Kinase Dilution Buffer only) samples (30 μ L) and controls (30 μ L) to the appropriate wells.
- Initiate reaction by adding 10 μ L of diluted ATP to each well (except blank). Incubate at 30°C for 90 min.
- Stop reaction by emptying contents of each well.
- Add 40 μ L of Phosphospecific Substrate Antibody to each well (except blank). Incubate at RT for 60 min.
- Wash wells 4 times with 100 μ L 1X Wash Buffer.
- Add 40 μ L of diluted anti-rabbit IgG-HRP conjugate to each well (except blank). Incubate at RT for 30 min.
- Wash wells 4 times with 100 μ L 1X Wash Buffer.
- Add 60 μ L TMB Substrate to each well. Incubate RT 30 – 60 min. Incubation time should be determined by the researcher according to color development.
- Add 20 μ L Stop Solution 2 to each well.
- Measure plate at OD 450 nm.

16.Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17.FAQs

Q. What is the active PKC concentration?

A. Because of the nature of the source (see above), the concentration of the protein can vary from lot to lot. Please see vial label for specific lot concentration.

18. Notes

Technical Support

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For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)