ab323608 – Adenylate Cyclase Activity Assay Kit (Colorimetric)

For the measurement of Adenylate Cyclase enzymatic activity in various samples.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab323608 (use www.abcam.cn/ab323608 for China, or www.abcam.co.jp/ab323608 for Japan)

Storage and Stability: Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt if components have not been reconstituted.
Reconstituted components are stable for 2 months.
Aliquot components in working volumes before storing at the recommended temperature.
Avoid repeated freeze-thaws of reagents.

Materials Supplied:

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer 15	25 mL	-20 °C	4°C or -20 °C
ATP IV	2 vials	-20 °C	-20 °C
Enzyme Mix X	200 µL	-20 °C	-20 °C
Developer Mix A	1 vial	-20 °C	-20 °C
AC Substrate Mix	200 µL	-20 °C	-20 °C
AC Positive Control	50 µL	-20 °C	-20 °C
OxiRed™ Probe	0.2 mL	-20 °C	-20 °C
Pyrophosphate Standard	200 µL	-20 °C	-20 °C

Materials Required, Not Supplied
Microplate reader capable of measuring absorbance in kinetic mode
Clear 96 well plate with flat bottom
Dounce Homogenizer
Ammonium Sulfate Solution (saturated, 4.32 M), ab273568

Reagent Preparation: Briefly centrifuge small vials at low speed prior to opening.

1. **Assay Buffer 15**: Warm to room temperature and ensure particulates have returned to solution before use. Buffer may be stored at 4°C or -20 °C after initial thawing.
2. **ATP IV:** Reconstitute each vial with 110 µL dH2O when needed. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months. Keep on ice while in use.
3. **Enzyme Mix X:** Store at -20°C, thaw and keep on ice while in use.
4. **Developer Mix A and AC Substrate Mix:** Reconstitute each vial with 220 µl dH_2O. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months. Keep on ice while in use.
5. **AC positive Control:** Reconstitute with 100 µL of 30% Glycerol and mix thoroughly. Aliquot and store at –20°C. Use within two months. Keep on ice while in use.
6. **OxiRed™ Probe:** Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. Keep at room temperature during the assay. Store at -20°C and **protect from light and moisture**. Once the probe is opened and thawed, it is stable for at least 3 additional freeze/thaw cycles but should be used within two months. After use, promptly retighten the cap to minimize adsorption of airborne moisture.
7. **Pyrophosphate Standard:** Provided as 1 mM stock solution. Store at -20°C, stable for at least 3 freeze/thaw cycles.

Sample Preparation

1. For whole cells or tissue lysate, rapidly homogenize tissue (50 mg) or cells (4 x 10^6) with 300 µl ice cold Assay Buffer 15, and place on ice for 10 minutes.
2. Centrifuge at 10,000 X *g* for 5 min and collect the supernatant in a clean microfuge tube.
3. Use the ammonium sulfate precipitation method to remove small molecules that could cause interference: aliquot homogenate supernatant (100 µl) to a clean centrifuge tube, add saturated ammonium sulfate to 65% saturation (1 volume of sample + 2 volumes of 4.32 M ammonium sulfate) mix and place on ice for 30 mins. Spin down samples at 10,000 x *g* at 4°C for 10 mins, discard the supernatant.
4. Resuspend the pellet back to the original volume with Assay Buffer 15.
5. For each sample, prepare parallel wells; one well will serve as a sample background control. Add 2-50 µl of sample to each of the wells in a clear 96-well plate and adjust the volume to 50 µl/well with Assay Buffer 15.
6. For AC Positive Control, dilute the reconstituted AC Positive Control stock 5-fold by adding 20 µL of AC Positive Control to 80 µl AC Assay Buffer. Add 5-20 µl of diluted Positive Control and adjust the final volume to 50 µl with Assay Buffer 15. Once diluted, the AC Positive Control should be kept on ice and used within 2 hours.

ΔNOTE: For unknown samples, we suggest testing several doses to ensure the readings are within the standard curve range. We recommend measuring sample protein concentration using the Bradford reagent or a comparable protein assay in order to calculate specific activity.

Pyrophosphate Standard Preparation
Always prepare a fresh set of standards for every use & discard working standard dilutions after use as they do not store well.

1. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM Pyrophosphate Standard into a series of wells.
2. Adjust the volume to 50 µL/well with Assay Buffer 15 to generate 0, 2, 4, 6, 8, and 10 nmol/well of pyrophosphate.

ΔNOTE: To improve accuracy and reduce CVs, the standard curve may be prepared in duplicate, in a microplate or microcentrifuge tubes using the table below. Each standard mix has enough volume to set up duplicate readings (2 x 50 µL). After mixing, 50 µL final volume must be added to the well.

Standard #	1 mM Pyrophosphate Standard (µL)	Assay Buffer 15 (µL)	Final volume standard in well (µL)	Pyrophosphate in well (nmol)
1	0	125	50	0
2	5	120	50	2

3	10	115	50	4
4	15	110	50	6
5	20	105	50	8
6	25	100	50	10

Assay Procedure

1. Make enough AC Reaction Mix and Background Control Mix for the number of assays to be performed, 50 µl per reaction as shown below. Remember to account for the Standard Curve wells when calculating the amount of Reaction Mix needed.

Component	AC Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer 15	40	42
ATP IV	2	2
Enzyme Mix X	2	2
Developer Mix A	2	2
AC Substrate Mix	2	-
OxiRed™ Probe	2	2

2. Add 50 µL of the AC Reaction Mix to Each well containing the Pyrophosphate Standard, AC Positive Control, and Test Sample(s). Gently mix by pipetting up and down
3. Add 50 µL of the Background Control Mix to each Sample Background Control well. The total reaction volume should be 100 µL/well. Gently mix by pipetting up and down.
4. Immediately begin measuring the absorbance at 570 nm in kinetic mode for 15-60 min at 37°C. We strongly recommend reading in kinetic mode in order to ensure that the measurements recorded are within the linear range of the reaction. Ideal measurement time for the linear range may vary depending upon the sample.

ΔNOTE: The Pyrophosphate Standard Curve wells may be read in endpoint mode (OD at 570 nm).

Data analysis

1. For the Pyrophosphate Standard Curve, subtract the 0 nmol/well Standard absorbance reading from all the Standard readings (ΔOD_{570} = $OD_{standard}$ – OD_{blank}). Plot the background-corrected Standard values and calculate the slope of the Pyrophosphate Standard Curve.
2. For Sample Reaction Wells (including paired Sample Background Control wells), choose any two time points (T_1 and T_2) in the linear phase of the reaction progress curves. Obtain the corresponding absorbance values at those points (A_1 and A_2) and determine the change in absorbance over the time interval: $\Delta A = A_2 - A_1$.
3. Subtract the Sample Background Control (ΔA_{BC}) from the corresponding Sample (ΔA_S) to obtain the net change in absorbance: $\Delta A_{NET} = \Delta A_S - \Delta A_{BC}$.
4. The net values (ΔA_{NET}) are applied to the Standard Curve to get *B* nmol of substrate metabolized during the reaction time.
5. To determine the Sample Adenylate Cyclase Activity, use the following equation:

$$\textbf{Sample Adenylate Cyclase Activity} = \frac{\boldsymbol{B}}{\boldsymbol{\Delta T \times V}} \times \boldsymbol{D} = nmol/(min * mL) = mU/mL$$

Where: $\boldsymbol{B}$ is the pyrophosphate amount(nmol) from Standard Curve
$\boldsymbol{V}$ is the sample volume (mL) added to the well
$\boldsymbol{D}$ is the sample dilution factor (if applicable, *D* = 1 for undiluted samples)
$\boldsymbol{\Delta T}$ is the change in time (T_2-T_1) for the sample

ΔNOTE: Remember to account for any dilution of the sample made during sample preparation, before adding sample to the sample well, when determining *D*.

Unit Definition: One unit of Adenylate Cyclase is the amount of enzyme that converts 1 µmole of ATP to cAMP, generating 1 µmole of Pyrophosphate per min at pH 7.0 and 37 °C.

Technical Support

For all technical or commercial enquiries please go to:
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