Version v8f Last updated 6 November 2023

ab138880 cAMP Direct Immunoassays Kit (Fluorometric)

For the sensitive and accurate measurement of adenylate cyclase activity in cell extracts, tissue extracts and biological fluids.

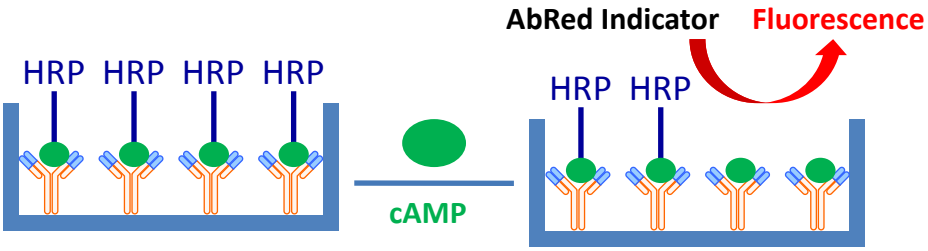
This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

cAMP Direct Immunoassay Kit (Fluorometric) (ab138880) provides a sensitive solution for detecting adenylate cyclase activity in biochemical or cell-based assays. The assay is based on the competition between HRP-labeled cAMP and free cAMP present in the sample for cAMP antibody binding sites. In the absence of cAMP, HRP-cAMP conjugate is bound to anti-cAMP antibody exclusively. Free cAMP (unlabeled) present in the test sample competes with HRP-cAMP for anti-cAMP antibody, therefore displacing HRP-cAMP and inhibiting its binding to the antibody. The fluorescence observed (Ex/Em = 540/590 nm) is proportional to the activity of HRP-cAMP conjugate: the more cAMP present in the test samples, the lower the fluorescence detected. This product can detect as low as 1 nM of cAMP present in the sample.

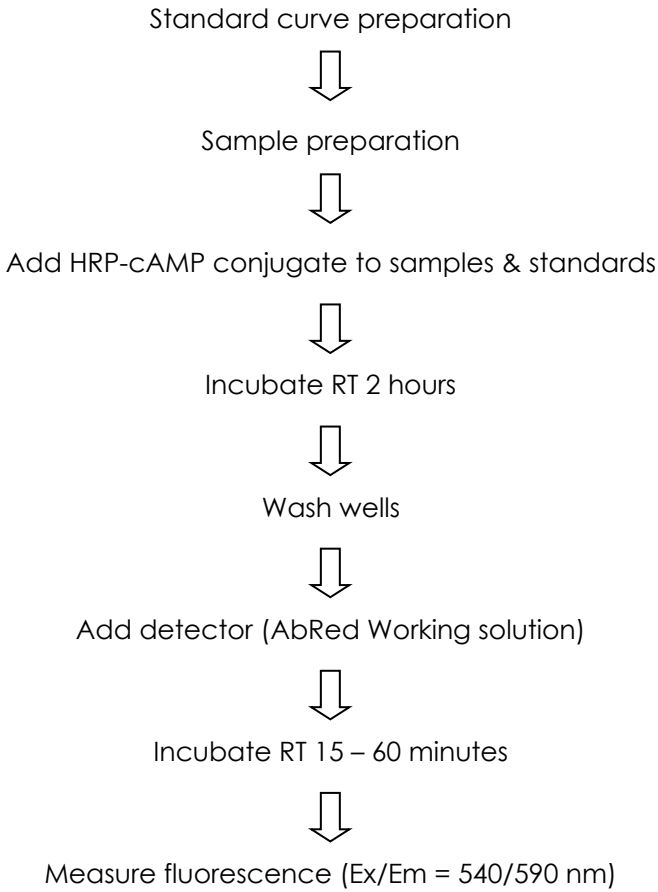
Compared to other ELISA cAMP assay kits, our kit eliminates the tedious acetylation step. The assay can be performed in a convenient 96-well or 384-well microtiter-plate and easily adapted to automation.



Maximum HRP activity
(no cAMP)

Decreasing HRP activity
(increasing free cAMP)

2. Protocol Summary



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 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)
	1 plate	10 plates		
cAMP Standard (lyophilized, 33 µg)	1 vial	1 vial	4°C	4°C
Assay Buffer	20 mL	100 mL	4°C	4°C
HRP-cAMP Conjugate	1 vial	1 vial	-20°C	-20°C
10X Wash Solution	10 mL	100 mL	4°C	4°C
Cell Lysis Buffer	10 mL	100 mL	-20°C	-20°C
Hydrogen Peroxide Solution (3% H_2O_2)	50 µL	250 µL	4°C	4°C
AbRed Indicator (lyophilized)	1 vial	1 vial	-20°C	-20°C
Substrate Buffer	10 mL	100 mL	4°C	4°C
Anti-c-AMP coated 96 well plate (black, flat bottom)	1 plate	10 plates	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 fluorescence at Ex/Em = 540/590 nm
- MilliQ water or other type of double distilled water (ddH_2O)
- 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
- Sterile 96 well plate with flat bottom for cell culture
- Reagents and instrumentation necessary to perform cell culture
- Dounce homogenizer (if using tissue)
- DMSO
- (Optional) Forskolin (ab120058) – to induce cAMP release

For suspension cells:

- Poly-D-lysine for plate coating to allow cell adhesion

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.
- 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.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- 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 Assay Buffer:
Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

9.2 cAMP Standard (33 µg):
Reconstitute standard in 1 mL of Assay Buffer to generate a 100 µM cAMP Standard stock solution. Aliquot cAMP standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.

9.3 HRP-cAMP Conjugate:
Reconstitute in 55 µL of Assay Buffer to generate a 50X HRP-cAMP Conjugate stock solution. Aliquot 50X HRP-cAMP conjugate stock solution so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.

Δ Note: for 10 x 96 tests kit – reconstitute in 550 µL of Assay Buffer to generate 50X Stock Solution.

9.4 10X Washing Solution:
Prepare **1X Washing Solution** by diluting 1 mL of 10X Washing Solution in 9 mL ddH_2O. Keep at room temperature while in use. Store unused 10X Washing Solution at 4°C.

9.5 Cell Lysis Buffer:
Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.6 Hydrogen Peroxide Solution:
Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

9.7 AbRed Indicator:
Reconstitute in 50 µL of DMSO (not included) to generate a 200X AbRed stock solution. Aliquot 200X AbRed stock solution so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.

Δ Note: for 10 x 96 tests kit – reconstitute in 500 µL of DMSO to generate 200X Stock Solution.

9.8 Substrate Buffer:
Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

9.9 Anti-cAMP coated 96 well plate:

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

Δ Note: if you are not using all wells in a single experiment, additional wells can be sealed prior the experiment for future use.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Use the 100 µM (100,000 nM) cAMP standard, prepare a standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Sample to dilute	Volume standard in well (µL)	Assay Buffer (µL)	End conc cAMP in well
1	100 µM stock	20	180	10,000 nM
2	Std #1	5	495	100 nM
3	Std #2	150	350	30 nM
4	Std #3	150	300	10 nM
5	Std #4	150	350	3 nM
6	Std #5	150	300	1 nM
7	Std #6	150	350	0.3 nM
8	Blank (none)	0	150	0 nM

Each dilution has enough amount of standard to set up duplicate readings (2 x 75 µL).

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- 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. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- Each cell line should be evaluated on an individual basis to determine the optimal cell density. Cells may be seeded the day before or on the day of the experiment depending on the cell type and/or the effect of the test compounds.

11.1 Adherent cell samples:

11.1.1 Grow 3 x 10^4 – 10^6 cells/well in a sterile 96-well plate. Incubate overnight in a 37°C/ 5% CO_2 incubator.
11.1.2 The following day, treat cells as required with test compound(s)of interest.
11.1.3 Aspirate cell solution after incubation.
11.1.4 Add 100 µL/well of Cell Lysis Buffer and incubate at room temperature for 10 minutes.
11.1.5 Centrifuge samples for 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
11.1.6 Collect supernatant and transfer to a new tube.
11.1.7 Keep lysate on ice.

11.2 Suspension cell samples:

11.2.1 Centrifuge growing cells and resuspend cell pellets in culture medium so that you can plate them at 3 x 10^4 – 10^6 cells/well in a sterile 96-well poly-D lysine coated-plate. Incubate overnight in a 37°C/ 5% CO_2 incubator.
11.2.2 The following day, treat cells as required with test compound(s)of interest.
11.2.3 Aspirate cell solution after incubation.

11.2.4 Add 100 µL/well of Cell Lysis Buffer and incubate at room temperature for 10 minutes.
11.2.5 Centrifuge samples for 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
11.2.6 Collect supernatant and transfer to a new tube.
11.2.7 Keep lysate on ice.

11.3 Tissue samples:

Δ Note: it is important to rapidly freeze tissues immediately after collection in liquid nitrogen due to the quick metabolism of cyclic nucleotides in tissue.

11.3.1 Weigh frozen tissue and add 10 – 20 µL/mg of Cell Lysis Buffer.
11.3.2 Homogenize tissue with a Dounce homogenizer with 10 – 15 passes on ice.
11.3.3 Centrifuge sample for 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
11.3.4 Collect supernatant and transfer to a new tube.
11.3.5 Keep on ice.

11.4 Plasma, Urine and Culture medium:

Urine: can be tested directly with 1:200 – 1:1000 dilutions in Cell Lysis Buffer.

Plasma: We recommend to use it undiluted.

Culture medium: can be tested directly with 1:10 – 1:200 dilutions in Cell Lysis Buffer.

Δ Note: RPMI medium may contain up to 350 fmol/µL cAMP.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

12.1 Plate loading:

12.1.1 Add standard and samples to wells of the anti-cAMP coated 96-well plate (Step 9.9) as follows:

- Standard wells = 75 µL standard dilutions.
- Sample wells = 5 – 75 µL samples (adjust volume to 75 µL/well with Assay Buffer).

12.1.2 Incubate plate at room temperature for 5 – 10 minutes.

12.2 cAMP Assay procedure:

12.2.1 Prepare 1XP HRP-cAMP conjugate working solution by diluting 1: 50 the 50X HRP-cAMP conjugate stock solution (Step 9.3) in Assay Buffer and mixing well. Keep on ice.

12.2.2 Add 25 µL/well of 1X HRP-cAMP conjugate to each standard and sample well.

12.2.3 Incubate plate at room temperature for 2 hours on a plate shaker.

12.2.4 Aspirate plate contents and wash plate 4 times using 200 µL/well of 1X Wash solution.

12.2.5 Prepare AbRed Working Solution by diluting 50 µL of 200X AbRed Stock solution (Step 9.7) and 11.5 µL of Hydrogen Peroxide solution (Step 9.8) into 10 mL of Substrate Buffer (Step 9.8).

Δ Note: AbRed Working solution is not stable. Do not store for future use.

12.2.6 Add 100 µL AbRed Working Solution into each standard and sample well. Mix well.

12.2.7 Incubate at room temperature for 10 min – 2 hours protected from light.

12.3 Plate measurement:

12.3.1 Monitor fluorescence increase at Ex/Em = 540/590 nm (cutoff 570 nm) using a microplate reader in top read mode.

13. Calculations

– Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

13.1 Calculate the mean value of the duplicate readings for each standard and sample.
13.2 To generate a Standard value, plot the graph using the standard concentrations on the x-axis and the corresponding mean fluorescence on the y-axis.
13.3 The best-fit line can be determined by regression analysis using a four-parameter logistic curve-fit.
13.4 Determine the unknown sample concentration from the standard curve and multiply the value by the dilution factor.

14. Typical Data

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

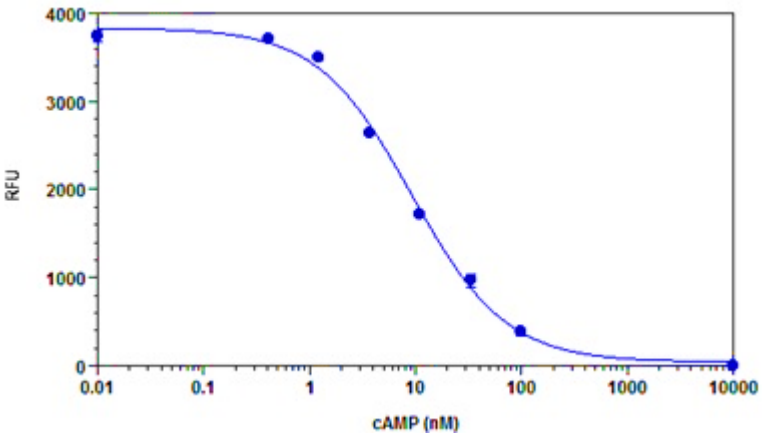


Figure 1. Typical cAMP dose response. Samples were measured in a solid black 96-well plate with a fluorescence microplate reader. The kit can detect as low as 1 nM cAMP in a 100 µL reaction volume.

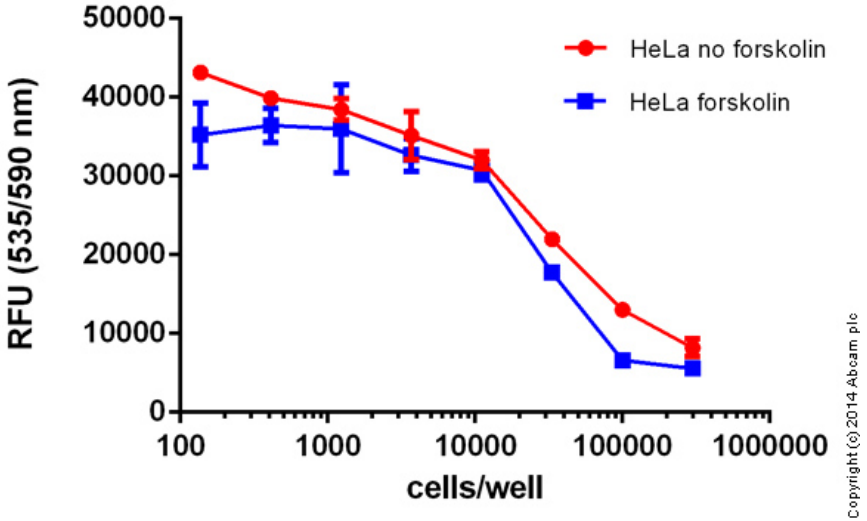


Figure 2. cAMP dose response. In increase amount of HeLa cells untreated or treated with 100 µM Forskolin (ab120058) for 15 minutes.

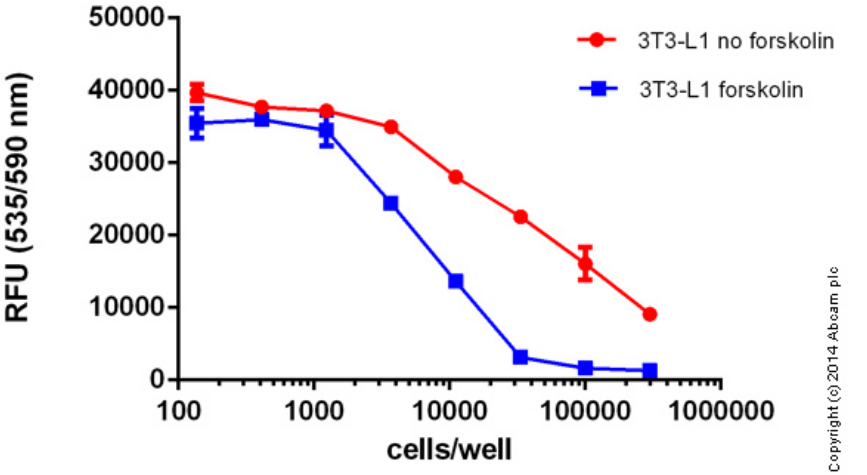


Figure 3. cAMP dose response. In increase amount of 3T3-L1 cells untreated or treated with 100 µM Forskolin (ab120058) for 15 minutes.

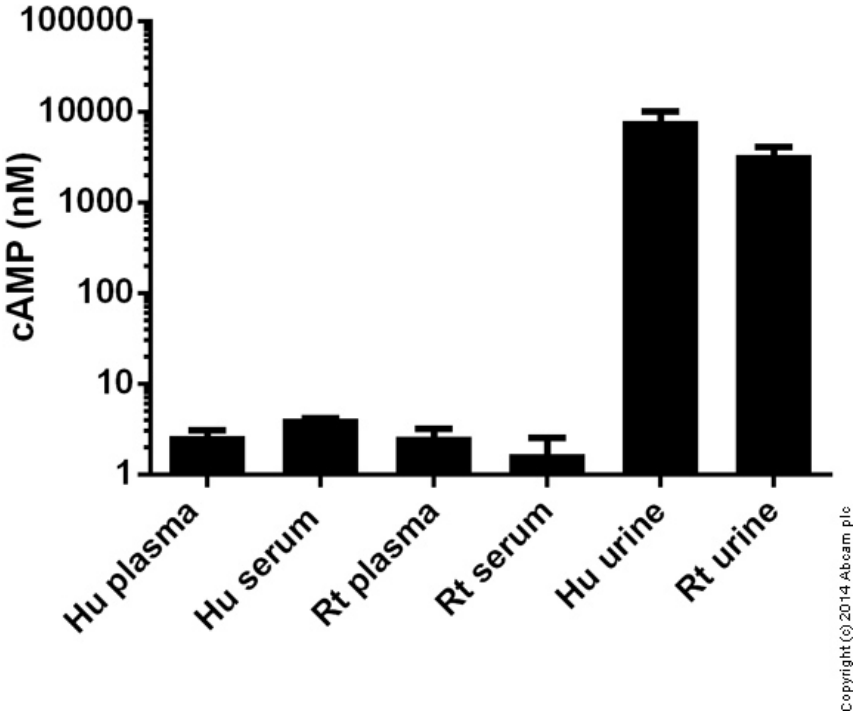


Figure 4. cAMP concentration (nM) measured in various biological fluids following assay protocol.

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 (aliquot if necessary); get equipment ready.
- Prepare cAMP standard dilution [0.3 nM – 10 µM].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (75 µL) and samples wells (75 µL).
- Add 25 µL 1X HRP-cAMP conjugate to wells.
- Incubate plate at RT for 2 hours.
- Wash plate 4 times with 200 µL Wash Solution.
- Add 100 µL AbRed Indicator Working Solution.
- Incubate plate at RT for 10 min – 2 hours protected from light.
- Monitor fluorescence change in a microplate reader set to top read mode at Ex/Em= 540/590 nm (cutoff 570 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
	Plate is insufficiently washed	Ensure enough time for proper wash. If using a plate washer, check all ports for obstructions
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

17. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

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