

LANCE[®] Ultra cAMP Kit

For Research Use Only

1. Intended use

The LANCE[®] Ultra cAMP kit is intended for the quantitative determination of 3',5'-cyclic monophosphate (cAMP) in cell culture and cellular membrane samples.

2. Provided reagents

Component	TRF0262 1,000 points*	TRF0263 10,000 points*	TRF0264 50,000 points*
cAMP standard, 50 μM	1 vial, 1 mL	1 vial, 1 mL	1 vial, 1 mL
Eu-cAMP tracer**	1 vial, 110 μL	1 vial, 1 mL	5 vials, 1 mL each
ULight™-anti-cAMP**	1 vial, 37 μL	1 vial, 340 μL	1 vial, 1.68 mL
cAMP Detection Buffer	1 bottle, 25 mL	1 bottle, 250 mL	4 bottles, 25 mL each
BSA Stabilizer (7.5% solution)	1 vial, 1 mL	1 bottle, 10 mL	1 bottle, 50 mL

* When using the recommended protocols (20-μL assay in 384-well microplates).

** Centrifuge tubes for a few seconds before use to improve recovery of content.

3. Storage conditions

Upon receiving the kit, store all reagents at 2-8°C protected from light. The expiration date of the kit is indicated on the box label.

4. Assay principle

The LANCE Ultra cAMP assay is a homogeneous time-resolved fluorescence resonance energy transfer (TR-FRET) immunoassay designed to measure cAMP produced upon modulation of adenylyl cyclase activity by G-protein coupled receptors (GPCRs). The assay is based on the competition between the europium (Eu) chelate-labeled cAMP tracer and sample cAMP for binding sites on cAMP-specific monoclonal antibodies labeled with the ULight™ dye. When antibodies are bound to the Eu-labeled cAMP tracer, light pulse at 320 or 340 nm excites the Eu chelate molecule of the tracer. The energy emitted by the excited Eu chelate is transferred by FRET to ULight molecules on the antibodies, which in turn emit light at 665 nm. Residual energy from the Eu chelate will produce light at 615 nm. In the absence of free cAMP, maximal TR-FRET signal is achieved (Figure 1, left panel). Free cAMP produced by stimulated cells competes with the Eu-cAMP tracer for the binding to the ULight-mAb, causing a decrease in TR-FRET signal (Figure 1, right panel).

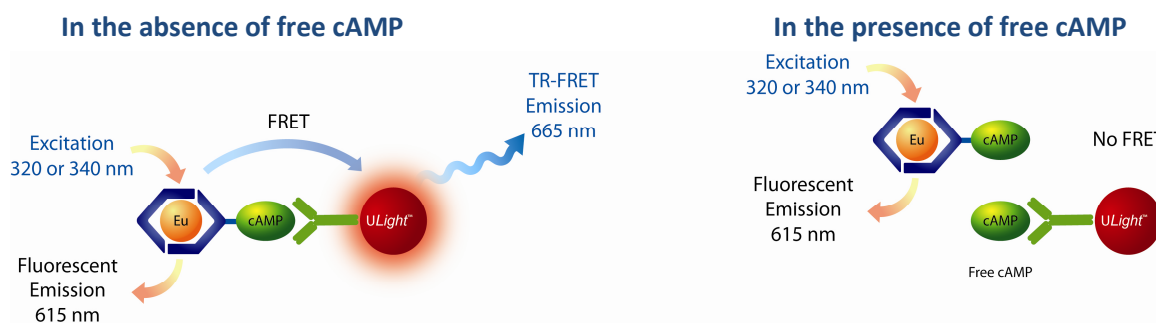


Figure 1. LANCE[®] Ultra cAMP assay principle

5. Reagents not supplied in the kit

Item	Recommended source	Product no.
Hank's Balanced Salt Solution (HBSS) (1X) (no phenol red)	Invitrogen	14025-092
HEPES Buffer Solution (1 M) pH 7.2 to 7.5	Invitrogen	15630-080
Forskolin	Sigma	F6886
IBMX	Sigma	I7018
OptiPlate-384, white	PerkinElmer	6007290 (pack of 50) 6007299 (pack of 200)
ProxiPlate-384 Plus, white	PerkinElmer	6008280 (pack of 50) 6008289 (pack of 200)
OptiPlate-96, white	PerkinElmer	6005290 (pack of 50) 6005299 (pack of 200)
OptiPlate-1536, white	PerkinElmer	6004290 (pack of 50) 6004299 (pack of 200)
TopSeal™-A 384	PerkinElmer	6005250

6. Assay optimization guidelines

The following protocol assumes that both the cell number and stimulation conditions have been optimized, as these parameters often vary for each receptor and cell line. It is therefore strongly recommended to generate either forskolin (Gs and Gi receptors) or full agonist (Gs receptors) concentration-response curves in order to determine the optimal cell number per well. We suggest testing from 250 to 5,000 cells per well in a 20- μ L assay. The optimal cell number will be the one for which the forskolin or agonist concentration-response curve covers most of the dynamic range of the cAMP standard curve. This typically corresponds to the cell density giving the highest signal to background (S/B) ratio calculated using the maximal signal (untreated cells) and the minimal signal obtained with a saturating concentration of agonist or forskolin (fully activated cells). From the example presented below (Figure 2), the cell concentration selected for subsequent experiments (ex. agonist dose-response curves) would be 2,000 cells/well. Additional assay development guidelines are available on PerkinElmer's website (www.perkinelmer.com).

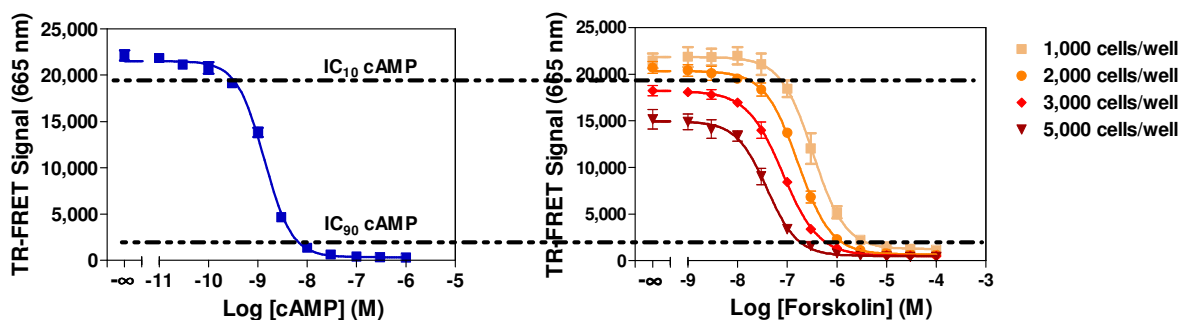


Figure 2. cAMP standard curve and forskolin cell titration

Reagent preparation

6.1 Preparation of Stimulation Buffer

The recommended Stimulation Buffer for cell-based assays is **1X HBSS, 5 mM HEPES, 0.5 mM IBMX, 0.1% BSA (pH 7.4)**. Make fresh.

NOTES:

- For cAMP standard curves, addition of 0.5 mM IBMX to the Stimulation Buffer is optional.
- We strongly recommend using the BSA Stabilizer (7.5% solution) included in the kit, as it is a highly purified preparation of BSA, free of europium and heavy metal ion contaminants.

To make 15 mL of Stimulation Buffer **1X HBSS containing 5 mM HEPES, 0.5 mM IBMX, 0.1% BSA (pH 7.4)**, add the following to a tube:

- 14 mL of 1X HBSS (Invitrogen, cat. # 14025-092)
- 75 μ L of 1M HEPES (Invitrogen, cat. # 15630-080)
- 15 μ L of 500 mM IBMX (Sigma, cat.# I7018)
- 200 μ L of 7.5% BSA Stabilizer (included in the kit)
- Adjust pH to 7.4 with 0.1N NaOH and complete volume to 15 mL with 1X HBSS

6.2 Preparation of cAMP standard serial dilutions in Stimulation Buffer

Prepare the **4X cAMP standard serial dilutions** in Stimulation Buffer from the 50 μ M cAMP standard supplied with the kit, as indicated in the table below.

Dilution	[Final] (M)	[4X] (M)	Volume of dilution	Stimulation Buffer
1	1×10^{-6}	4×10^{-6}	8 μ L of 50 μ M cAMP	92 μ L
2	3×10^{-7}	1.2×10^{-6}	30 μ L of 1	70 μ L
3	1×10^{-7}	4×10^{-7}	30 μ L of 2	60 μ L
4	3×10^{-8}	1.2×10^{-7}	30 μ L of 3	70 μ L
5	1×10^{-8}	4×10^{-8}	30 μ L of 4	60 μ L
6	3×10^{-9}	1.2×10^{-8}	30 μ L of 5	70 μ L
7	1×10^{-9}	4×10^{-9}	30 μ L of 6	60 μ L
8	3×10^{-10}	1.2×10^{-9}	30 μ L of 7	70 μ L
9	1×10^{-10}	4×10^{-10}	30 μ L of 8	60 μ L
10	3×10^{-11}	1.2×10^{-10}	30 μ L of 9	70 μ L
11	1×10^{-11}	4×10^{-11}	30 μ L of 10	60 μ L
12 (ctrl)	0	0	-	70 μ L

6.3 Preparation of Eu-cAMP tracer solution in cAMP Detection Buffer

Prepare a **4X Eu-cAMP tracer working solution** by making a **1/50** dilution of the Eu-cAMP tracer stock solution in cAMP Detection Buffer.

Example: Add 5 μ L of the Eu-cAMP tracer stock solution to 245 μ L of cAMP Detection Buffer and mix gently.

6.4 Preparation of *ULight*[™]-anti-cAMP solution in cAMP Detection Buffer

Prepare a **4X *ULight*-anti-cAMP working solution** by making a **1/150** dilution of the *ULight*-anti-cAMP stock solution in cAMP Detection Buffer.

Example: Add 5 μ L of the *ULight*-anti-cAMP stock solution to 745 μ L of cAMP Detection Buffer and ***mix gently***.

NOTES:

- Working solutions can be stored up to 24 hours at 4°C.
- For optimal assay performance, **do not** modify the recommended dilutions for both the Eu-cAMP tracer and *ULight*-anti-cAMP.

7. Assay protocols for a 384-well plate (total assay volume of 20 μ L)

In the protocols described in the table below, both the cells and tested compounds must be prepared in Stimulation Buffer (including 0.5 mM IBMX). cAMP Detection Buffer must be used only for the preparation of Eu-cAMP tracer and *ULight*-anti-cAMP working solutions.

cAMP standard curve	Gs Agonist	Gs Antagonist	Gi Forskolin titration	Gi Agonist	Gi Antagonist
5 μ L cAMP standard	5 μ L cell suspension	5 μ L cell suspension	5 μ L cell suspension	5 μ L cell suspension	5 μ L cell suspension
5 μ L Stimulation Buffer	5 μ L Agonist	2.5 μ L Agonist	5 μ L Forskolin	2.5 μ L Forskolin	2.5 μ L Forskolin/Agonist
-	-	2.5 μ L Antagonist	-	2.5 μ L Agonist	2.5 μ L Antagonist
Incubate 30 min at room temperature (optional step for cAMP standard curve)*					
5 μ L 4X Eu-cAMP tracer working solution					
5 μ L 4X <i>ULight</i> -anti-cAMP working solution					
Incubate 1 h at room temperature*					
Read on a TR-FRET microplate reader. Remove microplate seal prior to reading					

* Cover microplate with a TopSeal[™]-A film (PerkinElmer, Inc. Cat. # 6005250) or another plate during incubations.

NOTES:

- Additional readings can be performed for at least 24 hours after addition of LANCE *Ultra* reagents without significant change in assay sensitivity.
- If preferred, in order to eliminate one addition step, 5 μ L of cell suspension in Stimulation Buffer containing 4X *ULight*-anti-cAMP can be used. In this specific case, 10 μ L of 2X Eu-cAMP tracer solution must be added in order to keep the 20- μ L total assay volume.
- For 96- and 1536-well formats, adjust volume of each assay component proportionally in order to maintain the volume ratios used for the 384 plate format.
- Do not mix reagents from kits with different lot numbers in order to maintain assay performance between lots.

8. Instrument Settings

Parameter	VICTOR™	EnVision® Lamp/Laser	ViewLux®*
Flash Energy Area	High	N/A	N/A
Flash Energy Level	150	100%	600,000
Excitation Filter	320 / 340	UV2 320	DUG11 (UMB, AMC)
Integrator Cap	3	N/A	N/A
Integrator Level	2X LANCE High Count 615 and 665 (locked protocols)	N/A	N/A
Emission Filter	1) 615 2) 665	1) 203 - Eu 615 2) 205 - APC 665	1) 618/8 (Eu) 2) 671/8 (LANCE)
Delay Time	50 μ s	50 μ s	50 μ s
Readout Speed, Gain and Binning	N/A	N/A	Medium, High and 2x
Number of Flashes	N/A	Lamp: 100 Laser: 20	N/A
Window	100 μ s (200 μ s**)	100 μ s (200 μ s**)	354 μ s
Mirror Module	N/A	462 (D400/D630) or 412 (D400)	N/A
Cycle	2000 μ s	Lamp: 2000 μ s Laser: 16600 μ s	N/A

* Measurement time of **20 seconds** recommended for the ViewLux® instrument.

** If signal too low with 100 μ s.

9. Typical LANCE® Ultra cAMP standard curves

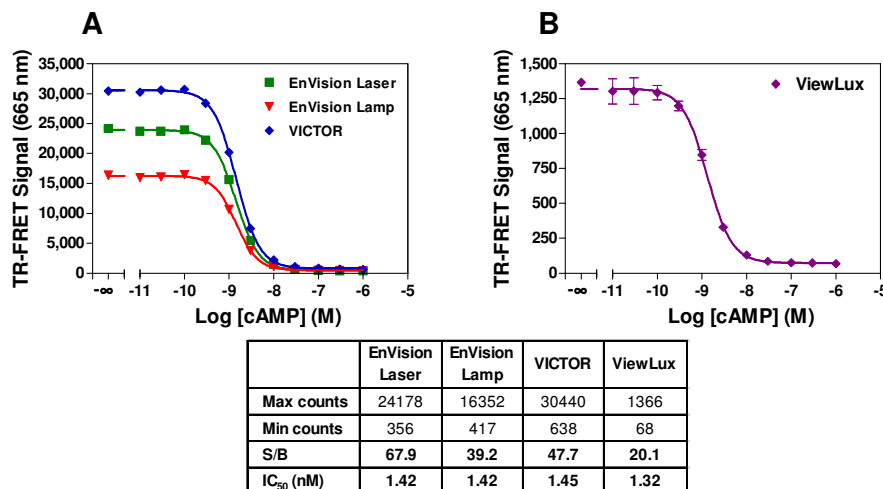


Figure 3. Representative LANCE Ultra cAMP standard curves obtained on different instruments using the recommended settings. A white OptiPlate™-384 microplate with a single cAMP standard curve assay was incubated for 1 hour at room temperature and then read with the (A) EnVision® Multilabel reader (laser and lamp settings), VICTOR™ reader and (B) ViewLux®.

NOTE: Depending on the instrument, counts and S/B ratio may vary, but this will not affect significantly assay robustness or sensitivity (IC₅₀).

For technical/application assistance, please contact PerkinElmer technical support:

PerkinElmer Life and Analytical Sciences
Direct Dial U.S. 800-762-4000
Toll Free Europe 00800-33290000
For Finland please dial 999 800 33 29 0000
E-mail: global.techsupport@perkinelmer.com
Please visit www.perkinelmer.com for specific country contact details.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
Phone: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com



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