

Cellul'erk - 200 tests Phosphorylated ERK1/2 detection using HTRF[®] technology HTRF[®] cell-based assay

Document reference : 64ERKPEF rev 00 (January 2010)

HTRF[®] package insert

Conjugate and buffer frozen set
Cellul'erk frozen set: 64ERKPEF

For in vitro research use only
Storage temperature: at ≤ -20°C

1. Introduction

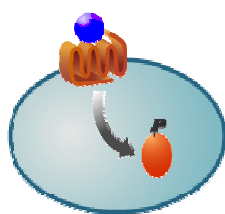
Cellul'erk enables simple, rapid and direct detection of phosphorylated extracellular signal regulated kinase (ERK1/2) in cells upon activation of a number of cell surface receptors.

A large variety of GPCRs may in particular activate the ERK signaling cascade. Given the importance of both upstream activators and downstream targets of ERK1/2, the control of ERK1/2 phosphorylation is crucial when studying the effect of an effector on a GPCR.

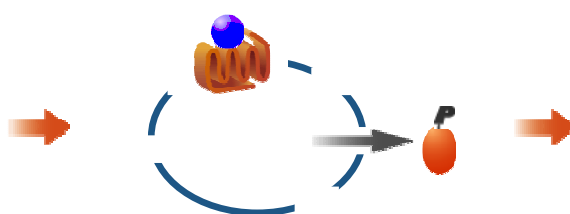
The assay using the truly homogeneous HTRF technology is antibodies based. This kit offers a new solution for high throughput screening of both agonists and antagonists of GPCRs expanding Cisbio Bioassays' offer for GPCRs investigation.

2. Assay description

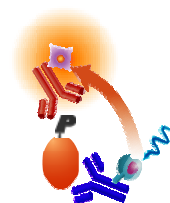
The HTRF assay is based on a sandwich immunoassay using an anti-phospho-ERK1/2 antibody labeled with d2 and an anti-ERK1/2 antibody labeled with Eu3+- Cryptate



GPCR activation by ligand induces ERK1/2 phosphorylation



Lysis of the cell induces the release of phosphorylated ERK1/2



Detection of phosphorylated ERK1/2 with HTRF conjugates

3. Reagents description - storage and stability

Supplied reagents	200 tests
Low control (Erk unstimulated cell lysate)	1 X 150 µL frozen – ready to use
High control (Erk Stimulated cell lysate)	1 X 150 µL frozen – ready to use
Erk lysis buffer (stock solution 4x)	2 X 2 mL frozen – to dilute
Erk blocking reagent (lysis buffer supplement)	1 X 130 µL frozen – to dilute
Erk detection buffer	1 X 2 mL frozen – ready to use
Anti-Phospho ERK 1/2- d2 Stock solution	1 X 20 µL frozen – to dilute
Anti-ERK1/2 Eu ³⁺ - Cryptate Stock solution	1 X 20 µL frozen – to dilute

Storage: all components must be stored at - 20°C or below.

To avoid freeze/thaw cycles, aliquote stock solutions into disposable plastic vials for storage at -20°C or below. Reagents may be frozen and thawed once.

Thawed buffers can be stored at 2-8°C.

4. Preparation of the working solutions

The working solutions are prepared from stock solutions (§ 3) by the following instructions below:

Ligand	Dilute ligand stock solution using a serum free cell culture medium to prepare working solutions for activation step.
Low control	Ready to use.
High control	Ready to use.
Supplemented lysis buffer	Dilute 4 fold Erk lysis buffer stock solution with distilled water (e.g. take 1 mL of Erk lysis buffer stock solution and add it to 3 mL of distilled water. Mix gently). Then dilute 100 fold Erk blocking reagent stock solution in the above prepared lysis buffer (e.g. take 40 µL of Erk blocking reagent stock solution and add it to 3960 µL of diluted lysis buffer. Mix gently). <i>It is recommended to prepare the required amount of supplemented lysis buffer, just before running the assay.</i>
Anti-Phospho ERK1/2- d2	Dilute 20 fold with Erk detection buffer (e.g. take 10 µL of stock solution and add it to 190 µL of detection buffer. Mix gently).
Anti-ERK1/2 Eu ³⁺ - Cryptate	Dilute 20 fold with Erk detection buffer (e.g. take 10 µL of stock solution and add it to 190 µL of detection buffer. Mix gently).


Do not store working solutions- It is recommended to prepare the required amount of working solutions just before running the assay

5. Assay protocol

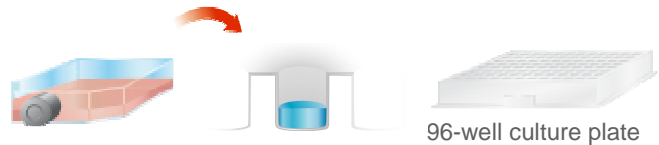
Day 1

Plate 50 μL of cells in 96-well tissue-culture treated plate and incubate overnight, at 37°C in CO₂ atmosphere.

1

 Dilute cells in growth medium using the appropriate cell density (cf. 6.1).

Depending on receptor a starving step with serum-free medium can be added.




96-well culture plate

Day 2

Dispense 50 μL of ligand and incubate at room temperature.

2

 Optimize the incubation time (cf. 6.2).



96-well culture plate

Remove carefully cell supernatant either by aspirating supernatant or flicking the plate.

3



96-well culture plate

Add **immediately** 50 μL of supplemented lysis buffer and incubate at room temperature with shaking.

4

 Optimize the lysis incubation time.



96-well culture plate

Transfer 16 μL of cell lysate from the cell-culture plate to a 384-well small volume white plate.

5



96-well culture plate

384-well SV white plate

Add 2 μL of each HTRF conjugates and incubate for 2 hours at room temperature (place a lid or a sealer on the plate during the incubation).

6



384-well SV white plate

Read the plate on a compatible reader (readouts must be performed at 620 nm for the donor and 665 nm for the acceptor).

5.1. Standard protocol in 20 μ L final volume (after lysis step)

	Non stimulated cell lysate	Stimulated cell lysate	Low control	High control	Blank control	Negative control
Non stimulated cell lysate	16 μ L	-	-	-	-	-
Stimulated cell lysate	-	16 μ L	-	-	-	-
Low control	-	-	16 μ L	-	-	-
High control	-	-	-	16 μ L	-	-
Supplemented lysis buffer	-	-	-	-	16 μ L	16 μ L
Erk detection buffer	-	-	-	-	2 μ L	-
Anti-phospho-ERK1/2-d2	2 μ L	2 μ L	2 μ L	2 μ L	-	2 μ L
Anti-ERK1/2 Eu ³⁺ - Cryptate	2 μ L	2 μ L	2 μ L	2 μ L	2 μ L	2 μ L
Total volume	20 μ L	20 μ L	20 μ L	20 μ L	20 μ L	20 μ L

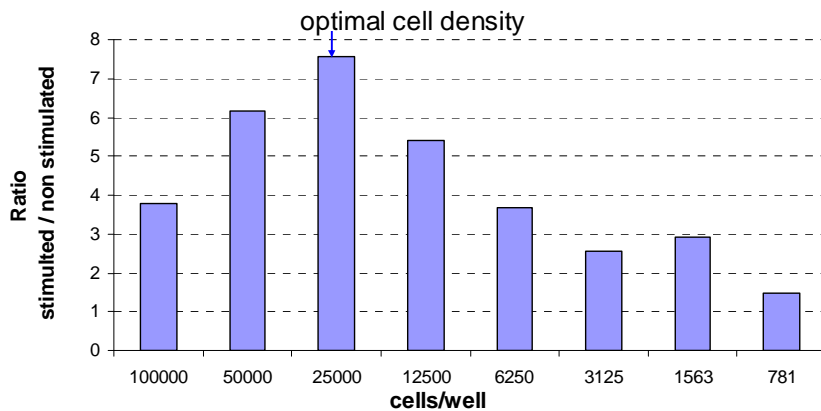
Blank control is used to check the Cryptate signal at 620 nm.

Negative control is used to check the Non specific signal.

6. Key parameters to optimize

Following graphs are given as an example.

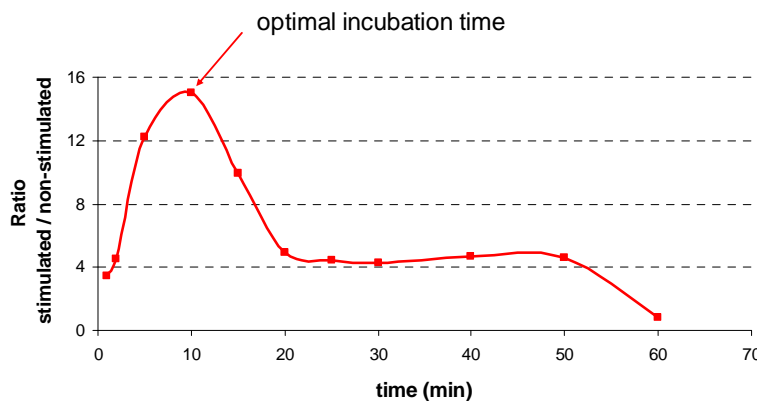
6.1. Cell density



Initial cell density has to be optimized in order to get the best assay window

*CHO-cell line
GPCR over expressed
Incubation time: 10min'*

6.2. GPCR activation time



Ligand incubation kinetic must be carefully studied

*CHO-cell line
GPCR over expressed
Cell density: 25,000*