

Cellul'erk – 10,000 tests – 64ERKPEH Phosphorylated ERK1/2 detection using HTRF[®] technology HTRF[®] cell-based assay

Document reference : 64ERKPEH rev 00 (January 2010)

HTRF[®] package insert

Conjugate and reagent sets

Cellul'erk frozen set: 64ERKPEH

Cellul'erk buffer set: 64ERKUDD

For in vitro research use only

Storage temperature: at $\leq -20^{\circ}\text{C}$

Storage temperature: 2-8 $^{\circ}\text{C}$

1. Introduction

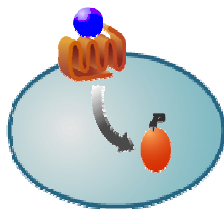
Cellul'erk enables simple, rapid and direct detection of phosphorylated extracellular signal regulated kinase (ERK1/2) in cells upon activation of a number of cell surface receptors.

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.

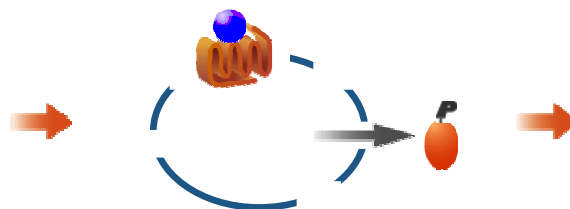
The assay using the truly homogeneous HTRF technology is antibodies based. This kit offers a new solution for high throughput screening of both agonists and antagonists of GPCRs expanding Cisbio Bioassays' offer for GPCRs investigation.

2. Assay description

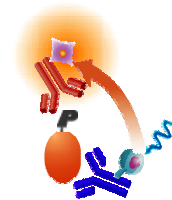
The HTRF assay is based on a sandwich immunoassay using an anti-phospho-ERK1/2 antibody labeled with d2 and an anti-ERK1/2 antibody labeled with Eu3+- Cryptate.



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 | 10,000 tests |
|---|----------------------------------|
| Low control (Erk unstimulated cell lysate) | 2 X 150 µL frozen – ready to use |
| High control (Erk stimulated cell lysate) | 2 X 150 µL frozen – ready to use |
| Erk blocking reagent (lysis buffer supplement) | 2 X 820 µL frozen – to dilute |
| Anti-Phospho ERK 1/2- d2 Stock solution | 1 X 200 µL frozen – to dilute |
| Anti-ERK1/2 Eu ³⁺ - Cryptate Stock solution | 1 X 200 µL frozen – to dilute |
| Erk lysis buffer (stock solution) | 1 X 50 mL liquid – ready to use |
| Erk detection buffer | 1 X 50 mL liquid – ready to use |

Storage: all frozen set 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.

The two buffers (Cellul'erk buffer set) must be stored at 2-8°C.

4. Preparation of the working solutions

The working solutions are prepared from stock solutions (§ 3) by 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 25 fold Erk blocking reagent stock solution in Erk lysis buffer stock solution (e.g. take 100 µL of Erk blocking stock solution and add it to 2400 µL of lysis buffer stock solution. Mix gently). <i>It is recommended to prepare the required amount of supplemented lysis buffer before running the assay.</i> |
| Anti-Phospho ERK1/2-d2 | Dilute 100 fold with Erk detection buffer (e.g. take 20 µL of stock solution and add it to 1980µL of detection buffer. Mix gently). |
| Anti-ERK1/2 Eu ³⁺ -Cryptate | Dilute 100 fold with Erk detection buffer (e.g. take 20 µL of stock solution and add it to 1980 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.

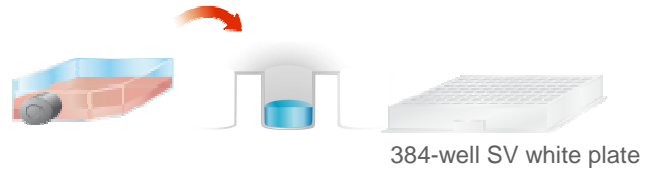
5. Assay protocol

Day 1

1 Plate 8 μL of cells in a 384-well small volume white plate (tissue culture treated) and incubate overnight, at 37°C in CO₂ atmosphere.


 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.




Day 2

2 Dispense 4 μL of ligand and incubate at room temperature.

 Optimize the incubation time (cf. 6.2).

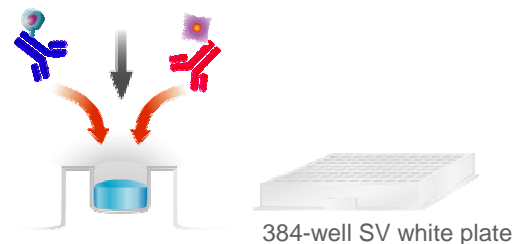


3 Add 4 μL of supplemented lysis buffer and incubate at room temperature with shaking.

 Optimize the lysis incubation time.



4 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).



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

5.1. Standard protocol in 20 μL final volume

| | Non stimulated cells | Stimulated cells | Low control | High control | Blank control | Negative control |
|---|----------------------|------------------|------------------|------------------|------------------|------------------|
| Cells | 8 μL | 8 μL | - | - | - | - |
| Serum free cell culture medium | 4 μL | - | - | - | - | - |
| Ligand | - | 4 μL | - | - | - | - |
| Low control | - | - | 16 μL | - | - | - |
| High control | - | - | - | 16 μL | - | - |
| Supplemented lysis buffer | 4 μL | 4 μL | - | - | 16 μL | 16 μL |
| Erk detection buffer | - | - | - | - | 2 μL | - |
| Anti-phospho-ERK1/2-d2 | 2 μL | 2 μL | 2 μL | 2 μL | - | 2 μL |
| Anti-ERK1/2 Eu ³⁺ - Cryptate | 2 μL | 2 μL | 2 μL | 2 μL | 2 μL | 2 μL |
| Total volume | 20 μL | 20 μL | 20 μL | 20 μL | 20 μL | 20 μL |

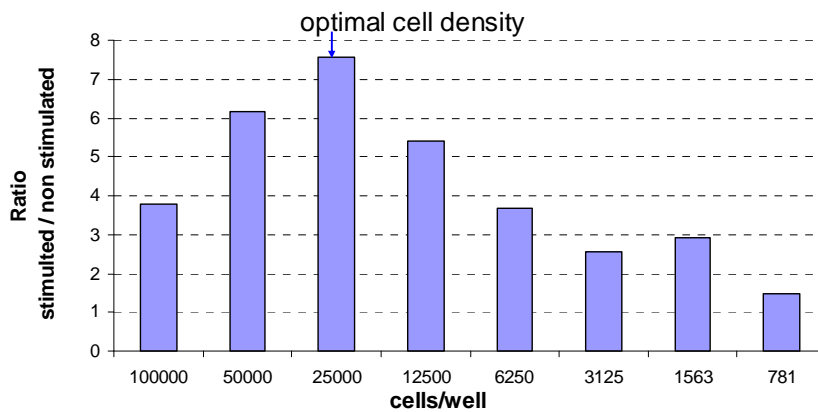
Blank control is used to check the Cryptate signal at 620 nm.

Negative control is used to check the Non specific signal.

6. Key parameters to optimize

Following graphs given as an example.

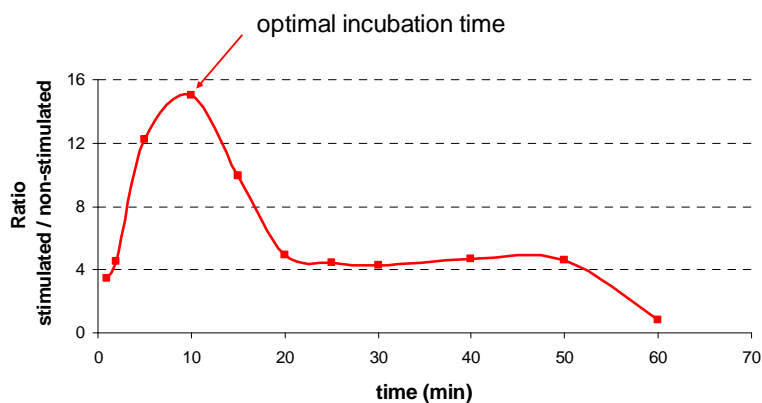
6.1. Cell density



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*