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Monitoring cell-surface expression of GPCR by ELISA

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ABSTRACT

Quantifying cell surface expression of G Protein-Coupled Receptors (GPCRs) can be extremely important for the expression of mutant receptors. Herein we report a useful Enzyme-Linked Immunosorbent Assay (ELISA) for the cell-surface detection of a HA-tagged version of a GPCR.

Protocol status: Working
We use this protocol and it's working

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21702

Keywords: G Protein-Coupled Receptor, Seven Transmembrane Receptor, Cell Surface Expression, ELISA, HA-tag, HRP-linked anti-HA

MATERIALS

MATERIALS

- ☒ NESTLE CARNATION Instant Non-fat Dry Milk **Contributed by users**
- ☒ Poly-L-lysine, 0.1% (wt/vol) **Sigma Aldrich Catalog #P8920**
- ☒ Falcon® 24-Well Flat-Bottom Plate, Tissue Culture-Treated 50 Plates **Stemcell Technologies Catalog #38021**
- ☒ HEK293 **ATCC Catalog #CRL-1573**
- ☒ Lipofectamine 3000 **Thermo Fisher Scientific Catalog #L3000015**
- ☒ Opti-MEM™ I Reduced Serum Medium **Thermo Fisher Catalog #31985062**
- ☒ Sterile Water **Wisent Bioproducts Catalog #809-115-CL**
- ☒ PBS 1X **Wisent Bioproducts Catalog #311-011-CL**
- ☒ Trypsin 0.25% / EDTA 2.21 mM in HBSS **Wisent Bioproducts Catalog #325-043-EL**
- ☒ SafeSeal tube 5mL **Sarstedt Catalog #72.701**
- ☒ Formaldehyde Reagent Grade **Bioshop Catalog #FOR201**
- ☒ 3 3'5 5'-Tetramethylbenzidine Liquid Substrate Supersensitive for ELISA **Sigma Aldrich Catalog #T4444-100ML**
- ☒ Anti-HA-Peroxidase High Affinity **Sigma Aldrich Catalog #12013819001**
- ☒ FBS (Fetal Bovine Serum) Premium Quality Endotoxin <1 Hemoglobin <25 **Wisent Bioproducts Catalog #080-150**
- ☒ DMEM 4.5g/L glucose with L-glutamine sodium pyruvate and phenol red **Wisent Bioproducts Catalog #319-005-CL**
- ☒ HEPES 1M Free acid **Wisent Bioproducts Catalog #330-050-EL**
- ☒ Penicillin (5000IU) / Streptomycin (5000µg/mL) sterile filtered for cell culture **Wisent Bioproducts Catalog #450-200-EL**

Endotoxin-free purified plasmidic DNA encoding for HA-tagged GPCRs

Tris-Buffered Saline (TBS, containing 20mM Tris-HCl pH 7.5 and 150mM NaCl)

Day 1 - Cell Culture & Transfections

- 1 **Coat 24-well plates with Poly-L-Lysine** (this need to be done in a biological safety cabinet to ensure sterility).

- 1.1 Add 300 µL of 0.1 mg/mL Poly-L-Lysine solution in each well of the 24-well plate and incubate 00:10:00 at Room temperature .

This can be done using a 300µL multichannel pipet fitted with 4 tips

1.2 Remove the Poly-L-Lysine solution (this solution can be re-used up to 4 times to coat cell culture plasticware).
This can be done using a 300µL multichannel pipet fitted with 4 tips

1.3 Rinse the wells twice with 300 µL of sterile water.

1.4 Let dry the 24-well plate without lid under the biological safety cabinet for 00:20:00 before seeding cells.

Note

Poly-L-Lysine-coated plates can be stored for several weeks at Room temperature before use.

2 Prepare transfections of plasmids encoding HA tagged-GPCRs (this need to be done in a biological safety cabinet to ensure sterility).

Note

In the case you want to compare expression of a mutant receptor, positive and negative controls are needed to normalize the results. The positive control should be the wild-type receptor and the negative control should be cells transfected with the empty vector (MOCK cells).

For 3 well of the 24-well plate:

2.1 Add 300 µL of Opti-MEM into a sterile 5mL tube.

2.2 Add 1.5 µg of plasmidic DNA encoding for the desired HA tagged-GPCR to the tube containing Opti-MEM and mix.

2.3 Add 3 μL of P3000 Reagent to the tube and mix.

2.4 Add 2.25 μL of Lipofectamine 3000 to the tube, mix, and incubate at Room temperature for 00:15:00 .

3 **Prepare HEK293 cells for transfection** (this need to be done in a biological safety cabinet to ensure sterility).

Note

Ideally, cells were seeded at a density of 3 million cells per 10cm-petri dish 48h before transfection to ensure a high transfection rate.

3.1 Remove culture media and rinse cells with PBS.

3.2 Add 1 mL of 0.25% Trypsin to a 10-cm petri dish and incubate for 00:02:00 at 37 °C .

3.3 Add 5 mL of complete DMEM (10% FBS, 20mM HEPES, Penicilin/Streptomycin) to the petri dish and dissociate cells by pipeting up and down.

3.4 Count cells using an automated cell counter or a hemacytometer.

Equipment

MOXI Z Mini

NAME

Automated Cell Counter

TYPE

Orflo

BRAND

MXZ001

SKU

https://www.orflo.com/product_p/mxz001.htm

LINK

- 3.5** Adjust cell concentration to 150,000 cells/mL.
- 3.6** Add 2 mL of the cell suspension at 150,000 cells/mL to the 5mL tube containing the plasmidic DNA and mix gently.
- 3.7** Dispense 450 µL of the mix of cell and plasmidic DNA to the desired wells of the Poly-L-Lysine-coated 24-well plate.
- 3.8** Incubate at 37 °C in humidified chamber at 5% CO₂ for 48:00:00 .

Day 3 - ELISA

- 4** **Detection of cell surface expression by ELISA** (this part of the protocol can be done on the wet bench).

Note

Buffer and reagents in this part of the protocol can be dispensed to the 24-well plate using combitips and a single channel repeater.

Equipment

M4 Repeater

NAME

Multidispense pipet

TYPE

Eppendorf

BRAND

4982000322

SKU

<https://online-shop.eppendorf.ca/CA-en/Manual-Liquid-Handling-44563/Manual-Pipetting-Dispensing-44564/Repeater-M4-PF-44619.html>

LINK

- 4.1 Remove cell culture media and wash each well with 400 µL of PBS.
- 4.2 Fix cells using 400 µL of 3.7 Mass Percent formaldehyde in TBS for 00:05:00 at Room temperature .
- 4.3 Rinse cells three times with 500 µL of TBS.
- 4.4 Block non-specific sites using 400 µL of 3 Mass Percent non-fat dry milk dissolved in TBS for 00:30:00 under gentle orbital agitation.

Note

In case non-fat dry milk is not suitable for blocking non-specific sites, a solution of [M] 1 Mass Percent of Bovine Serum Albumine in TBS can be used. The same solution must also be used for the incubation of the antibody.

- 4.5 Remove blocking solution and add 250 μ L of 1/1000 dilution of HRP-linked anti-HA antibody diluted in [M] 3 Mass Percent non-fat dry milk in TBS for 03:00:00 under gentle orbital agitation.

Note

This incubation step can also be done Overnight at 4 °C under gentle orbital agitation.

- 4.6 Remove antibody solution and wash each well three times with 500 μ L of TBS.
- 4.7 Add 250 μ L of Room temperature 3, 3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate, Supersensitive, for ELISA and incubate under gentle orbital agitation for 2 to 15 min (until the color of your positive control turn intense blue).
- 4.8 Stop the TMB reaction by adding 250 μ L of [M] 2 Molarity (M) Hydrochloric Acid (HCl).
- 4.9 Transfer 100 μ L of the colorimetric reaction to a flat-bottom transparent 96-well plate.
- 4.10 Read the absorbance at 450nm using a multimode plate reader.

Equipment

Mithras2 LB943

NAME

Multimode plate reader

TYPE

Berthold

BRAND

LB943

SKU

Results analysis

- 5 As this quantification of cell surface expression is a semi-quantitative method it should not be presented as raw OD_{450nm} values but rather as a percentage of expression compared to the positive control (or wild-type receptor).

To normalize the results, average the OD_{450nm} of the positive control and the OD_{450nm} of the negative control and apply the following formula:

$$y = \frac{x - \overline{x_{min}}}{\overline{x_{max}} - \overline{x_{min}}} \times 100$$

Normalization formula

y = normalized value

x = OD_{450nm} value of the sample

x_{min} = mean OD_{450nm} value of the negative control

x_{max} = mean OD_{450nm} value of the positive control