Mar 12, 2020

Monitoring cell-surface expression of GPCR by ELISA

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1 Works for me dx.doi.org/10.17504/protocols.io.zfef3je

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ABSTRACT

Quantifying cell surface expression of G Protein-Coupled Receptors (GPCRs) can be exteremly 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.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Gusach A, Luginina A, Marin E, Brouillette RL, Besserer-Offroy É, Longpré JM, Ishchenko A, Popov P, Patel N, Fujimoto T, Maruyama T, B Stauch, Ergasheva M, Romanovskaia D, Stepko A, Kovalev K, Shevtsov M, Gordeliy V, Han GW, Katritch V, Borshchevskiy V, Sarret P, Mishin A, Cherezov V. Structural basis of ligand selectivity and disease mutations in cysteinyl leukotriene receptors. Nature Communications. 2019; (10):5573. PMID: 31811124.

MATERIALS

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

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NAME	CATALOG #	VENDOR
HEPES 1M Free acid	330-050-EL	Wisent Bioproducts
Penicillin (5000IU) / Streptomycin (5000µg/mL) sterile filtered for cell culture	450-200-EL	Wisent Bioproducts

MATERIALS TEXT

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.

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

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

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 empy 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.

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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).

Ideally, cells were seeded at a density of 3 million cells per 10cm-pretri 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.

MOXI Z Mini
Automated Cell Counter
Orflo MXZ001

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** .

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Day 3 - ELISA

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

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

M4 Repeater
Multidispense pipet
Eppendorf **4982000322**

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 disolved in TBS for **00:30:00** under gentle orbital agitation.

In case non-fat dry milk is not suitable for blocking non-specific sites, a solution of **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 µl** of 1/1000 dilution of HRP-linked anti-HA antiboby diluted in **3 Mass Percent** non-fat dry milk in TBS for **03:00:00** under gentle orbital agitation.

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 µl** of TBS.

4.7 Add **250 µl** 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).

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4.8 Stop the TMB reaction by adding **250 µl** of **2 Molarity (M)** Hydroclhoric Acid (HCl).

4.9 Transfer **100 µl** of the colorimetric reaction to a flat-bottom transparent 96-well plate.

4.10 Read the absorbance at 450nm using a multimode plate reader.

Mithras2 LB943
Multimode plate reader
Berthold LB943

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

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