



ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE HUMAN RECOMBINANT GLP-2 GLUCAGON FAMILY RECEPTOR

HOST CELLS: Chem-11, a rat adherent cell line of hematopoietic origin expressing a proprietary recombinant promiscuous G-protein.

TRANSFECTION: Proprietary plasmids containing GLP2R cDNA encoding the GLP-2 Receptor (Accession Number: NM_004246; see CODING SEQUENCE below). The stable clonal cell line was selected by resistance to geneticin and Zeocin, followed by limited dilution cloning. The cell line was tested and found to have equivalent EC50 and signal at 1, 3 and 6 weeks of continuous culture.

PRESENTATION:

Cells are frozen at 2×10^6 cells/mL in 90% fetal bovine serum/10% DMSO. Cell line tests negative for mycoplasma.

STORAGE/HANDLING:

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37°C water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol. Transfer contents of the vial to a T75 flask containing Growth Media. Place the flask in a humidified incubator at 37°C with 5% CO₂.
3. After 8-24 h, all live cells will be attached. Viability of the cells is expected to be 50-80%. At this time, replace media to remove residual DMSO, and return to incubator.
4. When cells are approximately 80% confluent, passage the cells as follows: Remove media and wash once with HBSS without Ca⁺⁺ and Mg⁺⁺ (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37°C with 5% CO₂ until cells begin to round up and detach (5-10 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL Growth Media per 1 mL trypsin.
5. Cells are typically passaged 1:10 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.
6. Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count detached cells (prepared as in Step 4). Centrifuge cells at 200 x g for 5 min. Resuspend cells at 5×10^6 cells/mL in Freezing Media (cell densities of $2-10 \times 10^6$ are also acceptable if necessary). Dispense 1 mL aliquots into cryopreservation vials. Freeze the cells by a controlled rate process, such as in an isopropanol-jacketed container placed at -70°C overnight. Store the vials in liquid nitrogen.
7. Use of cells immediately after thawing is feasible for some cell lines and is being further validated. Some cell lines may need to be passaged at least once after thawing prior to use in calcium flux assays. Cells should be resuspended in Plating Media for plating for calcium assay.

MEDIA:

Chem-11 Growth Media:

- DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)
- 10% heat-inactivated FBS
- 1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
- 10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)
- 1x Pen-Strep (from 100x stock, Millipore TMS-AB2-C)



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250 µg/mL Genetecin/G-418
200 µg/mL Zeocin

Chem-11 Plating Media:

DMEM with 4.5 g/L glucose and 4 mM glutamine
10% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep

Chem-1 Freezing Media:

90% heat-inactivated FBS
10% DMSO (cell culture grade)

EXAMPLE ASSAY CONDITIONS:

1. Cells propagated for screening should be maintained and seeded at less than 90% confluency. Trypsinize cells as above and seed cells in 96-well black-walled, clear bottom plate at 50,000 cells/well in Chem-1 Plating Media. Keep the plate at room temperature for 1 h to allow even cell distribution in the plate, then transfer plate to a humidified incubator at 37°C with 5% CO₂.
2. Chem-1 derived cell lines have been successfully assayed using multiple commercially-available calcium dye kits following the manufacture's protocols. The protocol described below is a suggested protocol that can be generally applied to most calcium dyes kits.
3. Remove media
4. Wash cells with buffered salt solution
5. Add 100 µL/well calcium dye-loading solution.
6. Incubate the plate for 30 minutes in a humidified incubator at 37°C with 5% CO₂.
7. Incubate the plate for an additional 60 min at 25°C with 5% CO₂.
8. Set-up FLIPR to dispense 50µL/well 3X ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm (FLIPR^{TETRA}) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm (FLIPR^{TETRA}) or emission filter for Ca²⁺ dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height at 95 µL and dispense rate to 25 µL/sec. Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
9. Ligands are prepared in a white nonbinding surface 96-well plate (Corning 3605).
10. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

REFERENCES:

Dubé PE and Brubaker PL (2007) Frontiers in glucagon-like peptide-2: multiple actions, multiple mediators. *Am. J. Physiol. Endocrinol. Metab.* 293: E460-E465.

Munroe DG *et al.* (1999) Prototypic G protein-coupled receptor for the intestinotrophic factor glucagon-like peptide 2. *Proc. Natl. Acad. Sci. USA* 96: 1569-1573.



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CODING SEQUENCE:

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+1
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CTG CCC ATG GGC ATC CCT GCC CCC TGG GGG ACC AGT CCT CTC TCC TTC CAC AGG AAG TGC TCT CTC TGG 138
L P M G I P A P W G T S P L S F H R K C S L W 46
GCC CCT GGG AGG CCC TTC CTC ACT CTG GTC CTG CTG GTT TCC ATC AAG CAA GTT ACA GGA TCC CTC CTT 207
A P G R P F L T L V L L V S I K Q V T G S L L 69
GAG GAA ACG ACT CGG AAG TGG GCT CAG TAC AAA CAG GCA TGT CTG AGA GAC TTA CTC AAG GAA CCT TCT 276
E E T T R K W A Q Y K Q A C L R D L L K E P S 92
GGC ATA TTT TGT AAC GGG ACA TTT GAT CAG TAC GTG TGT TGG CCT CAT TCT TCT CCT GGA AAT GTC TCT 345
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GTA CCC TGC CCT TCA TAC TTA CCT TGG TGG AGT GAA GAG AGC TCA GGA AGG GCC TAC AGA CAC TGC TTG 414
V P C P S Y L P W W S E E S S G R A Y R H C L 138
GCT CAG GGG ACT TGG CAG ACG ATA GAG AAC GCC ACG GAT ATT TGG CAG GAT GAC TCC GAA TGC TCC GAG 483
A Q G T W Q T I E N A T D I W Q D D S E C S E 161
AAC CAC AGC TTC AAG CAA AAC GTG GAT CGT TAT GCC TTG CTG TCA ACC TTG CAG CTG ATG TAC ACC GTG 552
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GGA TAC TCC TTC TCT CTT ATC TCC CTC TTC CTG GCT CTC ACC CTC CTC TTG TTT CTT CGA AAA CTC CAC 621
G Y S F S L I S L F L A L T L L L F L R K L H 207
TGC ACG CGC AAC TAC ATC CAC ATG AAC TTG TTT GCT TCT TTC ATC CTG AGA ACC CTG GCT GTA CTG GTG 690
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AAG GAC GTC GTC TTC TAC AAC TCT TAC TCC AAG AGG CCT GAC AAT GAG AAT GGG TGG ATG TCC TAC CTG 759
K D V V F Y N S Y S K R P D N E N G W M S Y L 253
TCA GAG ATG TCC ACC TCC TGC CGC TCA GTC CAG GTT CTC TTG CAT TAC TTT GTG GGT GCC AAT TAC TTA 828
S E M S T S C R S V Q V L L H Y F V G A N Y L 276
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W L L V E G L Y L H T L L E P T V L P E R R L 299
TGG CCC AGA TAC CTG CTG TTG GGT TGG GCC TTC CCT GTG CTA TTT GTT GTA CCC TGG GGT TTC GCC CGT 966
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CTC AAA GCT CAT CAA ATG TGC TTC AGA GAT TAT AAA TAC AGA TTG GCA AAA TCA ACA CTG GTC CTC ATT 1173
L K A H Q M C F R D Y K Y R L A K S T L V L I 391
CCT TTA TTG GGC GTT CAT GAG ATC CTC TTC TCT TTC ATC ACT GAT GAT CAA GTT GAA GGA TTT GCA AAA 1242
P L L G V H E I L F S F I T D D Q V E G F A K 414
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TTT GCC AAT GGA GAG GTG AAG GCT GAG CTG CGG AAA TAC TGG GTC CGC TTC TTG CTA GCC CGC CAC TCA 1380
F A N G E V K A E L R K Y W V R F L L A R H S 460
GGC TGC AGA GCC TGT GTC CTG GGG AAG GAC TTC CGG TTC CTA GGA AAA TGT CCC AAG AAG CTC TCG GAA 1449
G C R A C V L G K D F R F L G K C P K K L S E 483
GGA GAT GGC GCT GAG AAG CTT CGG AAG CTG CAG CCC TCA CTT AAC AGT GGG CGG CTC CTA CAT CTA GCC 1518
G D G A E K L R K L Q P S L N S G R L L H L A 506
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M R G L G E L G A Q P Q Q D H A R W P R G S S 529
CTG TCC GAG TGC AGT GAG GGG GAT GTC ACC ATG GCC AAC ACC ATG GAG GAG ATT CTG GAA GAG AGT GAG 1656
L S E C S E G D V T M A N T M E E I L E E S E 552
ATC TGA 1662
I Stp 554

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