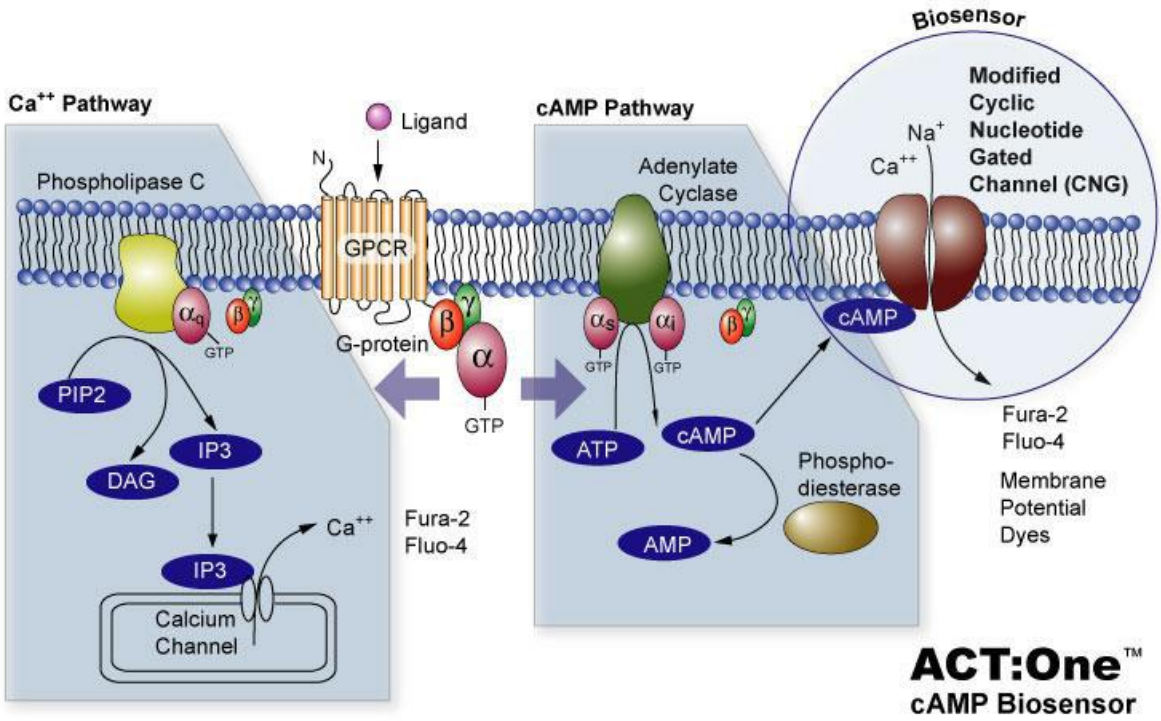
ACTOne™ Human C-C Chemokine Receptor Type 5 (CCR5) Stable Cell Line

Catalog Number: CL-11-CCR5
Product Type: Stable Cell Line
Cell Line Name: ACTOne™ CCR5
Host Cell Line: HEK-293 CNG
Target Receptor: Human C-C Chemokine Receptor Type 5 (CCR5)
GenBank Accession: NP_000570.1

Product Description

The ACTOne™ CCR5 stable cell line is a HEK-293 CNG derivative engineered to express recombinant human C-C chemokine receptor type 5 (CCR5). These cells contain a modified cyclic nucleotide-gated (CNG) channel that opens in response to intracellular cAMP elevation, triggering ion flux and membrane depolarization. This response is quantifiable using fluorescent membrane potential dye (Cat# CA-M165) or calcium-responsive dye (Cat# CA-C155).

The assay supports both endpoint and kinetic measurements of cAMP signaling and is compatible with FLIPR and fluorescence microplate readers.



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Applications

- Functional cAMP assays for Gi-coupled CCR5
- High-throughput screening of CCR5 agonists and antagonists
- GPCR pathway analysis in immunology, inflammation, and HIV research

Functional Validation

- **CCR5-specific response:** Confirmed
- **Post-thaw viability:** >2.5 million cells/vial on Day 2
- **Receptor activity stability:** Maintained over 10 weeks of continuous passage
- **Mycoplasma status:** Negative (tested lot-specific)

Product Format

- **Volume:** 1 mL
- **Cell Density:** 1x 10^6 cells/mL
- **Cryopreservation Medium:** 70% DMEM, 20% FBS, 10% DMSO

Growth Characteristics

- **Culture Type:** Adherent
- **Growth Medium:** DMEM + 10% FBS, supplemented with 250 µg/mL G418 and 1 µg/mL Puromycin
- **Freezing Medium:** 10% DMSO in complete growth medium

Subculturing Protocol

1. Thaw Cryovial in 37 °C water bath (1-2 min).
2. Decontaminate vial with 70% ethanol.
3. Transfer contents to a 75 cm^2 flask with 20 ml of complete DMEM growth medium.
4. Replace medium after 24 hours.
5. Remove and discard culture medium next day and then add fresh DMEM complete medium.
6. Monitor daily; passage at 90% confluence (1:3 split).
7. Use 0.25% trypsin-0.53 mM EDTA for detachment (15-20 min at 37°C).
8. Centrifuge at 250 x *g* for 5 to 10 min.
9. Resuspend and seed at 4-6 x 10^4 viable cells/cm^2.
10. Incubate at 37°C, 5% CO_2.

Freezing & Storage Instructions

1. Trypsinize cells and resuspend in freezing medium at 2.5 x 10^6 cells/mL.
2. Aliquot 1 mL per cryovial.
3. Freeze overnight at -80°C in a cryo container.
4. Transfer to vapor-phase liquid nitrogen (≤ -130°C) for long-term storage.

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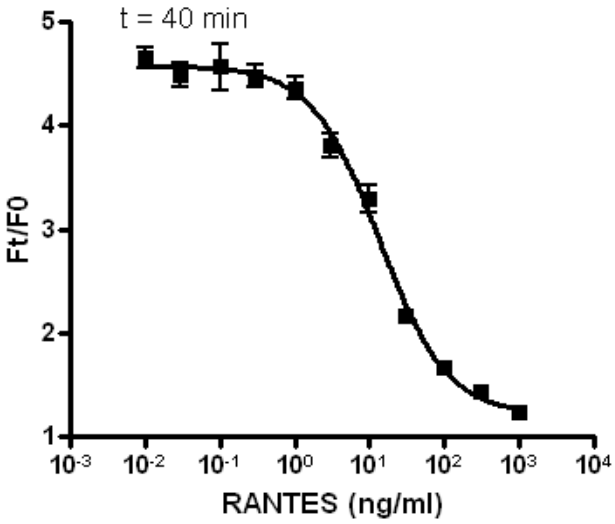
Data Summary

A



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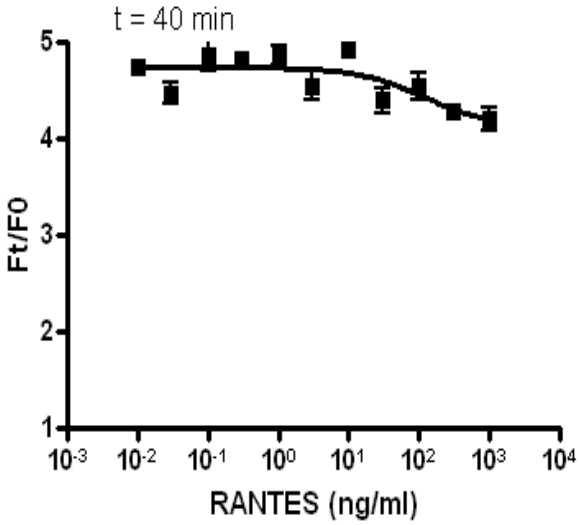

Figure 1. Response of ACT*One*™ CCR5 cell line & parental HEK-293 CNG cell line to RANTES.

ACTOne™ CCR5 cells and parental HEK-293 CNG cells were plated overnight in 20 µl culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 µl/well of 1x Dye-loading solution (membrane potential dye kit, Cat# CA-M165). After 2 hours of incubation at room temperature, two readings were obtained prior to and 30 min after the addition of RANTES. Ratios of the two readings (F/F0) are plotted in the figure.

A. **Dose response curve of RANTES in ACT*One*™ CCR5 cell line. EC50 = 13.5 ng/ml in the presence of PDE inhibitor Ro 20-1724 and adenosine agonist NECA.**
B. **Parental cells do not respond to RANTES.**

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