

Comparing Bioactivity Between Shenandoah CTGrade GMP rh IL-7 and Two Commercially-available Products

Technical note

Interleukin 7 (IL-7) is a member of the common (γ_c) chain cytokine family that also includes IL-2, IL-15, and IL-21. IL-7 signals through the IL-7 receptor to regulate the development and homeostasis of T cells, B cells, and NK cells.


FUJIFILM Irvine Scientific offers Shenandoah CTGrade GMP rh IL-7, a recombinant human protein produced from *E. coli*. It is designed to support preclinical and clinical research, as well as commercial applications.

The biological activity of CTGrade GMP rh IL-7 was determined in a PHA-stimulated human peripheral blood mononuclear cell (PBMC)-based proliferation assay and compared to two commercially-available rh IL-7 proteins.

PROTOCOL FOR IL-7 PROLIFERATION ASSAY

Complete medium: Iscove's Modified Dulbecco's Medium + 2 mM glutamine + 10% fetal bovine serum

Day 0

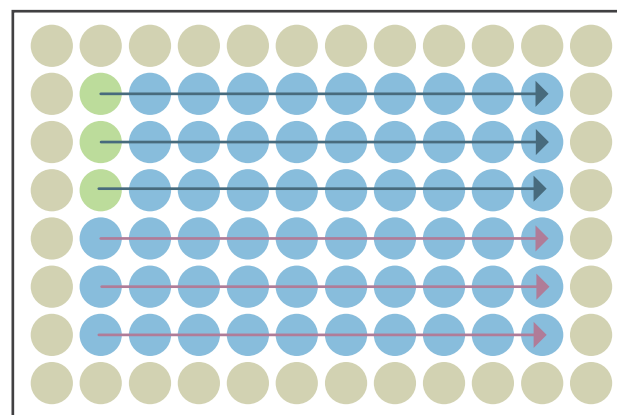
1. Thaw a fresh vial of cryopreserved human PBMCs by gentle agitation in a 37°C water bath. To reduce contamination risk, keep the cap of the vial out of the water.
2. Remove the vial from the water bath once the majority of the contents are thawed.
3. Decontaminate the vial with 70% isopropanol.
 *All procedures from this point should be performed under strict aseptic conditions.*
4. Carefully transfer the entire contents of the vial into a 10 mL conical tube containing pre-warmed 10 mL complete media.
5. Take an aliquot for cell counts.
6. Spin cells down at 125 xg for 5 minutes.
7. Discard the supernatant and resuspend to $2-5 \times 10^6$ cells/mL in fresh complete medium.
8. Incubate the culture overnight at 37°C and 5% CO₂.

Day 1

1. Take an aliquot of the cell suspension for cell counts.
2. Spin cells down at 125 xg for 5 minutes.
3. Discard the supernatant and resuspend the cells at 5×10^6 cells/mL in complete medium.
4. Activate the cells with 2.5 µg/mL phytohaemagglutinin (PHA) for 24 hours.

Day 2

1. After the 24-hour activation period, count the cells then centrifuge the cell suspension at 125 xg for 5 minutes.
2. Discard the supernatant, resuspend the cells in 1x PBS, then centrifuge the cell suspension at 125 xg for 5 minutes.
3. Repeat step 2, for a total of 2 washes with PBS.
4. Discard the supernatant and resuspend the cells to 1×10^5 cells/mL in the assay medium.
5. Dilute an aliquot of rh IL-7 to 600 ng/mL with the assay medium.
6. In a 96-well U bottom plate, add 150 µL of the diluted rh IL-7 to wells B2, C2 and D2, and add 100 µL of the assay medium or PBS to the other wells as shown in the plate diagram.
7. Serially dilute rh IL-7 by transferring 50 µL from one well to the next moving to the right using a multichannel pipette, as indicated by the arrows.
8. Add 100 µL of the PHA-stimulated PBMCs into each well.
9. Place the plate in a 37°C incubator with 5% CO₂ for 72 hours.



- Sample at 2X Starting Concentration
- Assay Medium
- PBS

1. After the 72-hour incubation period from Day 2, spin the plate at 300 xg for 2 minutes.
2. Carefully remove 100 μ L of supernatant from each well without disrupting the cell pellet.
3. Add 100 μ L of CellTiter-Glo into each well, pipette up and down, and transfer 100 μ L into a 96-well white flat bottom plate.
4. Read the luminescence using a suitable plate reader.

PERFORMANCE DATA

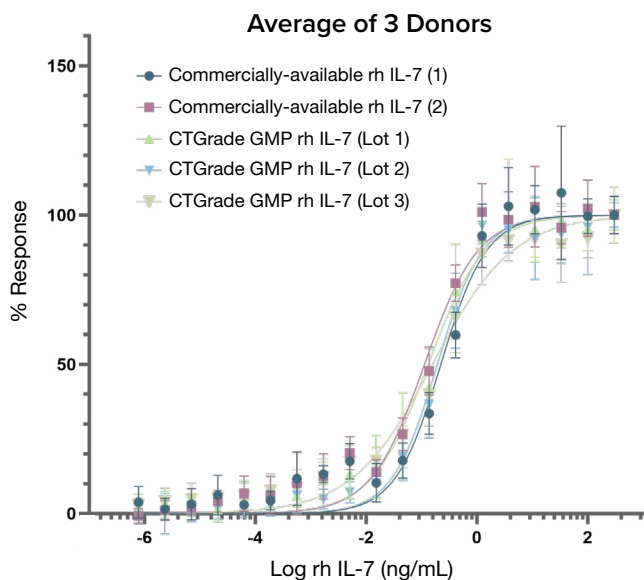


Figure 1. Dose-response curves and EC_{50} values of PHA-stimulated PBMCs treated with CTGrade GMP rh IL-7 compared to Commercially-available rh IL-7. Human PBMCs were activated with 2.5 μ g/mL PHA for 24 hours and cultured in serially diluted CTGrade GMP rh IL-7 (3 lots) or Commercially-available rh IL-7. These results are the average of the normalized response from 3 healthy donors, run in triplicate. Curves and EC_{50} values were generated from GraphPad Prism 9.

| EC_{50} (ng/mL) | Commercially-available rh IL-7 (1) | Commercially-available rh IL-7 (2) | CTGrade GMP rh IL-7 (Lot 1) | CTGrade GMP rh IL-7 (Lot 2) | CTGrade GMP rh IL-7 (Lot 3) |
|-------------------|------------------------------------|------------------------------------|-----------------------------|-----------------------------|-----------------------------|
| Donor 1 | 0.1554 | 0.09553 | 0.1130 | 0.1380 | 0.2040 |
| Donor 2 | 0.2795 | 0.1398 | 0.07261 | 0.1945 | 0.09886 |
| Donor 3 | 0.2511 | 0.1070 | 0.2713 | 0.2608 | 0.2091 |
| Average | 0.2229 | 0.1131 | 0.1322 | 0.1924 | 0.1580 |

CONCLUSION

Three lots of CTGrade GMP recombinant human interleukin-7 (Catalog# 500-07) were compared to rh IL-7 from two commercially-available proteins from three different PBMC donors. CTGrade GMP rh IL-7 showed similar or higher bioactivity in comparison to commercially-available rh IL-7, as determined by the calculated EC_{50} values.