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1. Description

Components	1 mL HIV Infectivity Enhancement Reagent.
Capacity	For 20 HIV infections.
Product format	HIV Infectivity Enhancement Reagent is supplied in a solution containing 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 HIV infectivity enhancement

Human immunodeficiency virus 1 (HIV-1) can be isolated and grown in primary human lymphocytes or immortalised cell lines. Often when working with primary isolates or recombinant replicating HIV, the titer of infectious virus present in the sample is low. It therefore requires considerable time to generate a high titer viral stock that can be used for subsequent experiments or only a small number of target cells can be infected for study. Virus isolates contain many more infectious virions than can be detected by standard infectious assay measurement.¹ A major problem therefore appears to be effective delivery of the virions to the target cell for their subsequent effective uptake by endocytosis² or fusion with the plasma membrane. The envelope of HIV-1 contains not only virus-encoded proteins, but also host cell proteins.^{3,4} These host cell proteins are incorporated either actively or passively when the virus buds from the cell membrane. Many of the cellular proteins present in the HIV envelope retain their biological function, suggesting that they could play a role in viral pathogenesis.³ The HIV Infectivity Enhancement Reagent uses the presence of these host proteins in both the virus envelope and the target cells to tether HIV on the surface of target lymphocytes and macrophages. Pre-treatment of viral isolates with HIV Infectivity Enhancement Reagent results in infection of a higher percentage of target cells, increased viral integration events, greatly enhanced

kinetics of viral replication and amplified viral titers and amounts of soluble p24 protein in the cell culture supernatant.⁵

1.2 Product applications

- Generation of high titer viral stocks from primary HIV isolates.
- Effective infection of target lymphocytes and macrophages with low titer HIV isolates for cellular and molecular assays.
- Expansion of primary HIV isolates for genomic and phenotypic characterization.
- Enhancement of sensitivity of tissue culture based assays that measure production of viral products.

▲ The HIV Infectivity Enhancement Reagent is not suitable for use with recombinant HIV that has been generated in HEK 293 cells.

1.3 Reagent and instrument requirements

- Replication-competent HIV originating from primary lymphocytes, macrophages or other cells expressing the CD44H isoform.
- T cell or macrophage cell lines, primary monocyte-derived macrophages, stimulated primary T cells or alternatively 3×3 stimulated PBMCs.
- Cell culture media and supplements.
- Polypropylene snapcap tubes (5 mL, 12×15 mm, and 14 mL, 17×100 mm), e.g., Falcon.
- MACSmix™ Tube Rotator (#130-090-753)

1.4 Related products

- μMACS VitalVirus HIV Isolation Kit (# 130-092-805)
- MultiMACS VitalVirus HIV Isolation Kit (12×8) (# 130-092-806)
- MultiMACS VitalVirus HIV Isolation Kit (4×96) (# 130-092-807)
- For 3×3 stimulation of PBMCs refer to www.miltenyibiotec.com/ protocols.
- For more information about MACSmolecular products and services refer to www.miltenyibiotec.com.

2. Protocol

HIV isolates that have been prepared in hematopoietic cell lines or in primary human hematopoietic cells are suitable for infection enhancement. Both fresh and frozen samples can be used. For efficient infection enhancement, target cells must also be of hematopoietic origin, such as T cells or macrophages. Effective enhancement of infection has been demonstrated using a wide range of ratios of infectious virus particles to target cells, multiplicities of infection (MOI) of 0.0005–0.5.

2.1 Generation of HIV enhancement complexes

1. Briefly centrifuge sample at 13,000×g for 30 seconds to remove particulate matter. Transfer supernatant to a fresh tube, avoiding floating fragments. Alternatively, filter through a 0.22 µm sterile filter.
2. Add 50 µL of HIV Infectivity Enhancement Reagent per 1 mL virus-containing sample.
3. Incubate at 4 °C for 30 minutes with gentle rotation on the MACSmix™ Tube rotator.
4. Proceed to section 2.2 for infection of suspension cells or section 2.3 for infection of adherent cells.

2.2 Enhanced infection of suspension cells

1. Transfer required number of target suspension cells to a suitable tube (2–10×10⁶ per 1 mL virus supernatant; see table 1). Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant.
2. Add virus complexes from section 2.1, step 3. Mix gently and incubate cells overnight (12–18 hours) at 37 °C in a cell culture incubator.
3. The next day, add 5–10 volumes of room temperature complete cell culture medium or PBS containing 2% serum. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant.
4. Resuspend cells in an appropriate volume of pre-warmed complete medium. Transfer cell suspension to a cell culture dish and incubate at 37 °C in a cell culture incubator.

Tube or cell culture plate	Cell number	Virus volume
5 mL (12×75 mm) polypropylene snapcap tube	≤ 5×10 ⁶	0.5 mL
14 mL (17×100 mm) polypropylene snapcap tube	5–15×10 ⁶	0.5–1.5 mL

Table 1: Recommended target cell numbers and HIV enhancement complex volumes for infection.

2.3 Enhanced infection of adherent cells

1. The day before infection, plate adherent target cells in a suitable cell culture dish at the cell densities recommended for your cell type and application. If working with primary monocyte-derived macrophages, we recommend plating monocytes at 3–5×10⁵ cells/cm² in complete medium supplemented with M-CSF 6 days before infection.
2. Directly before infection, remove the cell culture medium and add virus complexes from section 2.1, step 3. Incubate cells overnight (12–18 hours) at 37 °C in a cell culture incubator.
3. The next day, remove the medium and replace with fresh, pre-warmed complete cell culture medium.

2.4 Single-cycle infection of target cells

If a single-cycle infection of target cells with HIV is desired, then we recommend minimizing the contact times between virus complexes and target cells. For both adherent and suspension cells, the virus incubation step (sections 2.2 and 2.3, step 2) should not exceed 6 hours (1–6 hours). After incubation the cells should be washed extensively (4 times) with room temperature complete medium or PBS containing 2% serum before continued cultivation in pre-warmed complete medium. Analysis of the infected cells should take place before fresh infectious virus can be generated by the target cells (12–16 hours post-infection).

▲ **Note:** As an alternative, infection in the presence of HIV protease inhibitors will also lead to a single-cycle infection. Possible cytotoxic effects of the drug should be controlled for in the experimental design.

3. Example of enhancement of HIV infection kinetics in primary CD4⁺ T cells

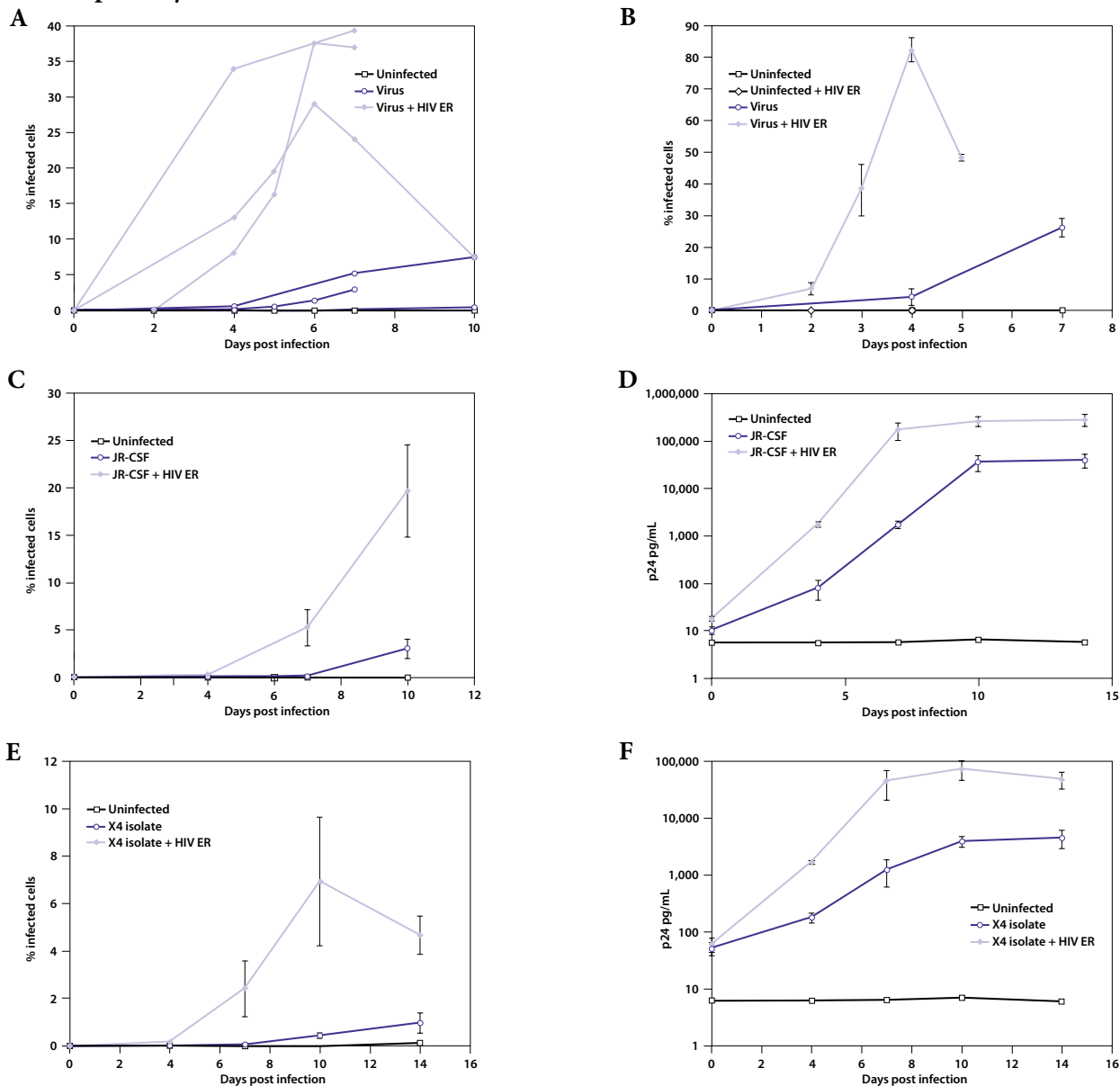


Figure 1: Infection of primary CD4⁺ T cells in the presence of HIV Infection Enhancement Reagent.

Primary CD4⁺ T cells (5×10^6) were infected overnight with MOI 0.05 IU of a GFP-expressing replication competent HIV clone (NL-EGFP; A) or 0.01 TCID₅₀ of the NL4-3 parental virus, $n=4$ (B). Infected cells were quantified by flow cytometry (GFP or intracellular p24). A marked increase in the numbers of infected cells and amount of secreted p24 can be seen after incubation with the HIV Infection Enhancement Reagent (+ HIV ER). The infections were so effective that cultures had to be terminated prematurely due to cellular cytotoxicity.

CD4⁺ T cells were also infected with an R5 clone, JR-CSF (C, D) and a primary X4 isolate 98IN017 (E, F) at an MOI of 0.01 pg p24 ($n=3$) with or without HIV Infection Enhancement Reagent pre-treatment. Infections were monitored by flow cytometry (intracellular p24) and by levels of secreted p24. Both levels of secreted p24 and the proportion of infected cells were increased following treatment with HIV Infection Enhancement Reagent (+ HIV ER) treatment. Error bars = SEM

4. Troubleshooting

No infection enhancement—HIV isolate not prepared in human hematopoietic cells, for example, primary CD4⁺ T cells, PBMCs, T cell lines). Prepare HIV isolate in human hematopoietic cells.

No infection enhancement—Target cells are not human hematopoietic cells, for example, primary CD4⁺ T cells, primary macrophages, PBMC, T cell lines. The HIV Infection Enhancement Reagent is only effective when human hematopoietic cells, expressing the CD44H molecule, are used as target cells.

5. References

1. Thomas, J. A. *et al.* (2007) Efficiency of Human Immunodeficiency Virus Type 1 Postentry Infection Processes: Evidence against Disproportionate Numbers of Defective Virions. *J. Virol.* 81: 4367–4370.
2. Miyauchi, K. *et al.* (2009) HIV Enters Cells via Endocytosis and Dynamin-Dependent Fusion with Endosomes. *Cell* 137: 433–444.
3. Tremblay, M. J. *et al.* (1998) The acquisition of host-encoded proteins by nascent HIV-1. *Immunol. Today* 19: 346–351.
4. Ott, D. E. (1997) Cellular proteins in HIV virions. *Rev. Med. Virol.* 7: 167–180.
5. Terry, V. H. *et al.* (2009) CD44 MicroBeads Accelerate HIV-1 Infection in T-Cells. *Virology* 388: 294–304.

All protocols and data sheets are available at www.miltenyibiotec.com.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Warranty

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