MACS molecular

Isolation of infectious HIV-1

from culture-derived virus or virus-containing sample

Special protocol

µMACS™ Streptavidin Kit Order no. 130-074-101

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1. Description

1.1 Background

The envelope of human immunodeficiency virus 1 (HIV-1) contains not only virus-encoded proteins, but also host cell proteins.[1,2] 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.[1] In addition, the presence of certain host cell type-specific antigens in the viral envelope serve as markers of the cellular origin of the virus particle.[3] MACS® Technology enables the rapid and efficient magnetic isolation of infectious HIV-1 virions from culture-derived HIV-1, human plasma or serum, and other bodily fluids, e.g. cerebral spinal fluid or cervical lavage.

It has been determined that CD44, expressed on all leukocytes, is the most effective host cell marker for the general labeling and capture of HIV-1 from patient samples and culture-derived virus, independent of the origin of the virus (lymphoid or myeloid cells).[4] The µMACS™ VitalVirus HIV Isolation Kit enables the direct isolation of HIV using CD44 MicroBeads. Virus originating from distinct subpopulations of leukocytes can be isolated indirectly using biotinylated antibodies directed against other cellular surface antigens in combination with µMACS Streptavidin MicroBeads.[4,5] Accordingly, virus originating from lymphoid cells (T cell-derived HIV) can be isolated with biotinylated CD26 antibodies; for virus of myeloid origin (macrophage-derived HIV-1) biotinylated CD36 can be employed.

This protocol describes a magnetic-based method to isolate viable and infectious HIV-1 for downstream studies utilizing biotinylated CD26 or CD36 antibody and the µMACS Streptavidin Kit. For isolation of HIV virions with CD44 marker, please refer to µMACS VitalVirus HIV Isolation Kit user manual.

1.2 Research applications

- Isolation of HIV-1 from patient samples for viral compartmentalization studies from either T cells, by using a biotinylated CD26 antibody, or from macrophages, by using a biotinylated CD36 antibody.
- Enrichment and detection of T cell- and/or macrophage-derived HIV-1 from normal, diluted, and/or difficult samples, e.g. cerebral spinal fluid, cervical lavage, breast milk. The µMACS™ Streptavidin Kit can be used in conjunction with commercially available kits for viral load analysis, see section 2.3, Elution option A.
- Enrichment of live and infectious T cell- and/or macrophage-derived HIV-1 for downstream studies, including neutralization studies, and the development of primary isolates, see section 2.4, Elution option B.
- Enrichment of T cell- and/or macrophage-derived infectious HIV-1 in the presence of neutralizing antibodies or other uncharacterized HIV serum inhibitors.

1.3 HIV-1 capture strategy

Isolation of T cell- and/or macrophage-derived HIV-1 from human patient sample and culture-derived virus is performed by positive selection using a biotinylated CD26 or CD36 antibody in combination with the µMACS Streptavidin Kit. First, virions are labeled with the antibody during a short incubation period, followed by the labeling of the HIV-1 virions using the µMACS Streptavidin MicroBeads. The magnetically labeled virions are enriched on a µ Column in the magnetic field of a µMACS Separator.

1.4 Reagent and instrument requirements

- µMACS Streptavidin Kit (# 130-074-101)
- Phosphate-buffered saline (PBS) pH 7.2, supplemented with 0.5% bovine serum albumin (BSA) or 2% fetal bovine serum (FBS)
- For enrichment of HIV of lymphoid origin: biotinylated CD26 antibody (clone 202.36 has been validated)
- For enrichment of HIV of myeloid origin: biotinylated CD36 antibody (clone SMO has been validated)
- µMACS Separator (# 130-042-602)
- MACS® MultiStand (# 130-042-303)
- (Optional) 5 mL syringe plunger for elution of intact virions
- (Optional) 0.5% Igepal CA-630 (formerly NP-40) in PBS-lysis buffer for viral antigen immunoassay[3] for in-column viral lysis and subsequent quantification, e.g. Innotest® HIV-1 Antigen mAb, Innogenetics Inc.
- (Optional) Lysis buffer from commercial viral load assay kit for in-column viral lysis and subsequent quantification, e.g. NucliSens® EasyQ viral load assay, bioMerieux, Inc.

Related products

- µMACS™ VitalVirus HIV Isolation Kit (#130-092-805)

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2. Protocol for isolation of HIV-1 virions

2.1 Magnetic labeling

Up to 1 mL virus-containing sample can be processed per isolation, e.g. plasma, serum, cerebral spinal fluid, breast milk, or culture supernatant. If fresh, non-frozen samples are used, virus isolation should be started as soon as possible after collection to minimize HIV-1 degradation.

1. For virus samples in saline solutions, e.g. vaginal lavage samples: add non-specific blocking reagent, i.e. 0.5% BSA or 2% FBS.
2. Briefly centrifuge sample, except breast milk samples, at 13,000×g for 30 seconds to remove particulate matter. Transfer supernatant to a fresh tube, avoiding floating fragments.

   ▲ **Note:** Do not centrifuge breast milk samples as milk will separate.
3. Add 1 µg of biotinylated CD26 or CD36 antibody per 200 µL virus-containing sample, i.e. plasma, serum, or culture supernatant, and incubate for 30 minutes at room temperature. For samples of less than 200 µL volume, also add 1 µg of biotinylated antibody.

   ▲ **Note:** Substitute CD26 or CD36 for isolation of compartmentalized HIV derived from T cells or macrophages, respectively.

   ▲ **Note:** For isolation of virus from samples containing high concentrations of natural biotin, e.g. breast milk, we recommend using 1.5 µg biotinylated antibody per 500 µL sample.[5]
4. Add 50 µL of µMACS™ Streptavidin MicroBeads per 200 µL of sample and incubate for 10 minutes at room temperature. For samples of less than 200 µL, adjust total volume to 200 µL with PBS containing 0.5% BSA or 2% FBS before adding 50 µL of µMACS Streptavidin MicroBeads.

   ▲ **Note:** For isolation of virus from samples containing high concentrations of natural biotin, e.g. breast milk, we recommend using 75 µL of µMACS Streptavidin MicroBeads or alternatively 100 µL of Anti-Biotin MicroBeads (# 130-090-485) per 500 µL sample.[5]

2.2 Magnetic separation

1. Place a µ Column in the magnetic field of the µMACS Separator that is mounted on the MACS® MultiStand.
2. Prepare the column by applying 100 µL of Equilibration Buffer for protein applications, supplied with the µMACS Streptavidin Kit, on top of the column.
3. Rinse columns with 3×100 µL PBS containing 0.5% BSA (or 2% FBS).
4. Add the magnetically labeled sample from section 2.1.4 to the column.
5. Wash the µ Column with 4×200 µL of PBS containing 0.5% BSA (or 2% FBS). Only add new buffer when the column reservoir is empty.

2.3 Elution option A for virion lysate

Perform an in-column viral lysis for downstream quantification by immunoassay or viral load determination. Buffer volumes should be adapted to the downstream quantification assay. For expected virus titres of $> 10^6$ virions per mL, a p24 antigen immunoassay is adequate. For expected titres of $< 10^6$ virions per mL, a more sensitive viral load assay is recommended. Examples are given below for the Innotest® HIV-1 Antigen mAb, Innogenetics Inc. and for the NucliSens® EasyQ viral load assay, bioMérieux, Inc.

2.3.1 Elution for viral antigen immunoassay

1. Add 100 µL of lysis buffer, e.g. 0.5% Igepal CA-630 in PBS, to the column. Incubate the column at room temperature for 5 minutes.
2. Add an additional 150 µL of lysis buffer and collect all drops.
3. Proceed to immunoassay.

2.3.2 Elution for viral load determination

1. Add 50 µL of lysis buffer, typically supplied in viral load assay kit, to the column. Collect flow-through. Incubate the column at room temperature for 5 minutes.
2. Add an additional 150 µL of lysis buffer and collect all drops.
3. Combine the eluates (total volume: 200 µL) and add 700 µL of lysis buffer. Proceed to viral load determination assay.

   ▲ **Note:** The NucliSens® EasyQ viral load assay requires 900 µL final volume and supplies 900 µL aliquots of buffer.

2.4 Elution option B for intact virions

Perform elution of virus with column outside of magnetic separator to obtain viable and infectious virus for downstream studies. HIV virions remain infectious even with MicroBeads attached.

1. Remove the column from the separator and place it on a suitable collection tube, e.g. a 1.5 mL tube. Add 200–500 µL of cell culture medium, or PBS, or other physiological buffer to the column, and collect the eluate containing the magnetically labeled virions.

   ▲ **Note:** To increase elution efficiency, apply pressure with 5 mL syringe plunger after adding media/buffer.

3. References

1. Tremblay, M. J. *et al.* (1998) The acquisition of host-encoded proteins by nascent HIV-1. Immunol. Today 19: 346–351.
2. Ott, D. E. (1997) Cellular proteins in HIV virions. Rev. Med. Virol. 7: 167–180.
3. Lawn, S. D. *et al.* (2000) Cellular compartments of human immunodeficiency virus type 1 replication in vivo: determination by presence of virion-associated host proteins and impact of opportunistic infection. J. Virol. 74: 139–145.
4. Lupo, L. D. and Butera S. T. (2004) Application of µMACS Streptavidin MicroBeads for the analysis of HIV-1 directly from patient plasma. MACS&more 8(1): 16–19.
5. Lupo, L. D. and Butera S. T. Unpublished observations.

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