

Immunophenotyping simultaneously with RT-PCR *in situ* hybridization, DNA-PCR *in situ* hybridization, and *in situ* hybridization

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Equipment and reagents

- ◆ Antibodies (Becton-Dickinson)
- ◆ PBS, pH 7.4 and 8.3 (see [Detection of single-copy HIV-1 DNA by PCR *in situ* hybridization](#))
- ◆ Permeafix (see [Detection of single-copy HIV-1 DNA by PCR *in situ* hybridization](#))
- ◆ Streptavidin-conjugated fluors (Becton-Dickinson)

Method

- 1 Adjust cell samples to a concentration of $1-2 \times 10^6$ cells per ml in PBS, pH 7.4.
- 2 Centrifuge the cells at 300–600*g* for 5 min.
- 3 Remove the supernatant and resuspend the cells in 90 μ l of PBS and 10 μ l of biotinylated anti-CD4 or CD14, for example.^a
- 4 Centrifuge the cells at 300–600*g* for 2 min and wash the cell pellet twice in PBS.
- 5 Fix and permeabilize the cells by resuspending with light vortexing in 50 μ l of Permeafix and incubate at ambient temperature for 60 min.
- 6 Centrifuge the cells as above, wash twice in 500 μ l PBS, pH 7.4, resuspend in PCR reaction mix and continue with [Detection of single-copy HIV-1 DNA by PCR *in situ* hybridization](#), [Detection of multiply spliced HIV-1 tat mRNA by RT-PCR *in situ* hybridization](#), or [Detection of single-copy HIV-1 DNA using Real-Time \(Taqman\) PCR *in situ*](#).
- 7 Following the last wash in [Detection of single-copy HIV-1 DNA by PCR *in situ* hybridization](#) and [Detection of multiply spliced HIV-1 tat mRNA by RT-PCR *in situ* hybridization](#) or following the post-FISNA PBS wash ([Detection of single-copy HIV-1 DNA using Real-Time \(Taqman\) PCR *in situ*, Step 7](#)), resuspend the cells in 80 μ l of PBS, pH 7.4, and 20 μ l streptavidin–phycoerythrin and incubate the mixture for 30 min at ambient temperature. Wash the cells in PBS, pH 7.4, as described above.
- 8 Centrifuge the cells as described and resuspend in PBS, pH 8.3, for analysis by flow cytometry or image analysis.^b

Notes

- a Dinitrophenol (DNP) conjugated antibodies are also thermostable.
- b Cells can be attached to slides for image analysis using a cytocentrifuge. A total of 2.5×10^4 – 1.0×10^5 cells is optimal for each spot on the slide.