



Testing lentiviruses for appearance of replication-competent retroviruses (RCRs)- safety tests

This service is mandatory before the concentrated vector prep is released to investigators. The concentrated HIV-vector preps are evaluated for the appearance of replication-competent retrovirus (RCR) by the three independent assays described below.

1. **Tat-transfer assay.** The assay described here is based on a reporter HeLa-CD4-LTR- β gal cells containing a copy of the HIV-1's LTR which is linked to the β -galactosidase gene. The reporter cell line is highly susceptible to the infection. In the case of viral genome recombination that results in the reconstitution of replication-competent HIV-1, the recombined vector will be capable to generate functional tat protein. The tat-expression will lead to activation of the viral LTR-promoter driven the expression of β -galactosidase gene of the reporter cell line. To execute the assay, lentiviral vectors contained supernatant is collected and serially-applied to fresh HEK293T cells four times (about two weeks in culture). Then, the supernatant is transferred to a reporter HeLa-CD4-LTR- β gal cells. The HIV-1-tat activity is determined by X-Gal (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside) staining. By this method, vector preparation is considered helper negative when no expression of β -gal is detected.
2. **Gag-transfer assay.** This assay is based on the detection of p²⁴gag-protein of the virus in conditioned media obtained from vector-transduced cells. The cells are serially passaged three-four times (about 2 weeks), after which the supernatant of the cells is collected for assessing the level of p24gag by ELISA (p24 ELISA kit, NIH). The detection limit of this method is ≥ 100 pg/mL of p²⁴, which is about 10^3 copies of viral genome per mL. By this method, vector preparation is considered helper negative when p24 is not detected.
3. **Marker-rescue assay.** This assay is based on the direct detection of GFP or other reporter following the transfer. Viral vector stocks are assessed as follows. The cells transduced with lentiviral vector harboring a reporter (GFP) are serially passaged four times, after which the supernatant of the cells is collected and transferred to HEK293T cells cultured in a 10-cm plate. Seventy-two hours' posttransduction, the cells are scored for a reporter expression. Vector stock was considered helper free when no reporter is detected.

References

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