Standard 4-hours Chromium-51 (^{51}Cr) Release Assay

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[Abstract] ^{51}Cr-release assays are commonly used for the precise and accurate quantification of cytotoxicity, particularly in the study of tumor cytolysis. This test has the advantage of requiring only very few cells.

Materials and Reagents

1. Target cells: Acute Myeloid Leukemia (AML) cell line (U937 or HL60 from ATCC) or AML cells isolated from patients with AML
2. Effector cells: γδ T cells isolated from Healthy volunteer provided by Etablissement
3. Francais du Sang (EFS) or patients
4. Medium : RPMI 1640 (Life Technologies, Gibco®)
5. Fetal calf serum (FCS) heat-inactivated 1 h at 56 °C
6. Chromium-51 (^{51}Cr, 5 mCi/ml) (PerkinElmer)
7. Complete medium = RPMI 1640 medium supplemented with 10% heat-inactivated FCS

Equipment

1. Centrifuge for microplates
2. Microplates (96 well round-bottom) for cell incubations
3. LumaPlate™ (PerkinElmer)
4. Liquid scintillation counter
5. Incubator

Controls

1. Spontaneous Release: Target cells were incubated alone (replace effector cells by 50 µl of media). After 4 h of incubation, wells were centrifuged and 50 µl of supernatant were recovered.
2. Maximum load: Target cells were incubated alone (replace effector cells by 50 µl of media). After 4 h of incubation, wells were mixed and 50 µl of cell suspension were

recovered rather than disrupt the cell membrane to release the radioactivity into the supernatant). This avoids the use of detergent.

Procedure

A. ^{51}Cr labeling the target cells
   1. Wash targets (2.106 per condition) in 15 ml of medium.
   2. Centrifuge targets; discard the supernatant and resuspend pellets in 20 µl of FCS.
   3. Add 100 µCi of ^{51}Cr (20 µl of stock solution at 5 mCi/ml) for one hour in an incubator at 37 °C.
      NB: To define the dose of chromium necessary to mark a cell type, it must be ensured that the spontaneous release is less than 10% of maximum load.
   4. Wash cells and resuspend in 60,000 cells per ml of complete medium.
   5. Add 3,000 Target cells per well in 50 µl of a round-bottom 96 well plate.

B. Incubation of target cells with effector cells
   1. Add effector cells (50 µl) at E: T (Effetor: Target) ratios of 30:1, 20:1, 10:1, and 1:1. In parallel, set up controls (Spontaneous Release, Maximum load) and incubate 4 h at 37 °C.

C. Analysis
   1. Centrifuge samples and collect supernatant (50 µl).
   2. Supernatants are dried on a LumaPlate™ (PerkinElmer) overnight and counted in a liquid scintillation counter.
      The percentage of specific lysis was calculated using the standard formula [(experimental - spontaneous release)/(maximum load- spontaneous release) x 100] and expressed as the mean of triplicate samples

Acknowledgments

This protocol is adapted from Gertner-Dardenne *et al*. (2012).

References

1. Gertner-Dardenne, J., Castellano, R., Mamessier, E., Garbit, S., Kochbati, E., Etienne, A., Charbonnier, A., Collette, Y., Vey, N. and Olive, D. (2012). Human Vgamma9Vdelta2 T cells specifically recognize and kill acute myeloid leukemic blasts. *J Immunol* 188(9): 4701-4708.