

Improve functional activities of cells in vitro through depletion of dying and dead cells

Principle

Dead-Cert® super-paramagnetic Nanoparticles are specially prepared to allow direct removal of dying and dead cells from cell suspensions and cultures with minimum fuss and maximum efficiency. The active components, stably coupled to the surface of these 250 nm particles, are proprietary molecules that can selectively bind structures that are exposed on cells during and following death.

Dead-Cert® Nanoparticles can be used directly in the natural environment of the cells. No columns are required for magnetic separation.

Using our novel technology, excellent 'clean-up' of cell populations can be achieved in under 1 hour with minimal cell manipulation - hence traumatization of cells leading to further death is minimized. **Dead-Cert®** clean-up boosts assay sensitivity because of the removal of inhibitory non-viable cells.

Procedure

If using cryopreserved cells, thaw cells rapidly according to appropriate protocol, wash and culture for at least 4 hours in order to allow cryo-stressed cells to die.

Prepare mono-disperse cell suspension in cell culture medium or **Dead-Cert® YES Medium (Yield-Enhancing Separation Medium, Cat. No. DC-004)**.

- 1 Note that protein-free media should be avoided. YES medium is recommended as a standard optimization medium.

Vortex the stock vial of **Dead-Cert® Nanoparticles** for 30 seconds immediately before use to ensure complete resuspension.

Note that aggregation of the particles inhibits their binding efficiency.

Transfer an aliquot of resuspended nanoparticles to a polypropylene tube containing cell culture medium or YES medium.

- 2 We recommend using a 1.5 ml tube containing 1 ml medium and 5-20 μl nanoparticles per $1-5 \times 10^6$ cells (adjust nanoparticle concentration up or down to suit cell number to be separated)

Mix well to wash particles.

Place the tube in the magnet rack for 3-5 minutes, remove medium, remove tube from magnet and thoroughly resuspend nanoparticles in 100 μl YES medium, fresh culture medium or buffer of choice.

- 3 Vortex or repeatedly pipette to resuspend nanoparticles but avoid creating air bubbles.

Add up to 5×10^6 cells in 100 μl YES medium, fresh culture medium or buffer of choice. Mix gently.

- 4

Place tube upright and incubate mixture for 40 minutes at $4^\circ - 8^\circ\text{C}$ to allow nanoparticles to bind to dead and dying cells.

- 5

Add 0.8 ml YES medium, fresh culture medium or buffer of choice.

Gently mix in single pipetting action and place on magnet.

After 3-5 minutes remove viable cell suspension.

Dead and dying cells with **Dead-Cert® Nanoparticles** bound are retained on the tube wall.

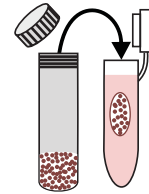
Viable cell suspension is ready for use in functional screening assay.

Wash and resuspend in appropriate culture medium.

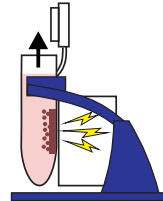
- 6



vortex nanoparticles



transfer aliquot & wash in culture medium

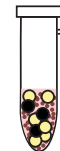


use magnet to recover nanoparticles

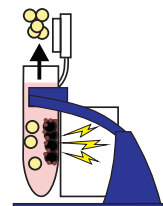
remove medium



add cells



incubate



use magnet to recover viable cells

This generic protocol may require minor modifications for optimal separation of different cell types: optimise by varying particle/cell ratio, temperature, incubation time and volume (see overleaf).

Note that **Dead-Cert® Nanoparticles** are effective in removing dead and dying cells from cell populations, sparing truly viable cells but, because they detect changes at the cell surface very early in the process of cell death, cells that appear 'viable' by other methods, but which are committed to die, are also removed.

For further information on all **Dead-Cert®** products, go to www.immunosolv.com

Optimising Dead-Cert® Nanoparticles

Improving purity and yields of viable cells



Dead-Cert® Nanoparticles are designed for use in depleting non-viable cells of many different eukaryotic cell types. Through recognition of conserved ligands exposed on dead and dying cells, **Dead-Cert® Nanoparticles** selectively bind to the altered surfaces of non-viable cells in a range of different cell culture media and can be used at temperatures ranging from melting ice to 37°C. While broadly different cell types from many different sources can be separated effectively using the **generic protocol** supplied with **Dead-Cert® Nanoparticles** or the complete **Dead-Cert® Nanoparticles Kit (DC-003)**, a simple optimisation regime to improve viable-cell purity and yield is required in some circumstances, depending on several variables including cell number, starting viability, culture medium requirements and phagocytic capacity of the cells undergoing separation. Follow the **simple steps** in these application notes to optimise depletion of non-viable cells in your cell system.

1. Separation Medium

Non-viable cells can be separated effectively in a variety of different culture media and in many cases the separation medium of choice is the cells' preferred culture medium, especially if it contains serum and does not cause cell clumping. In general, use of protein-free media tends to produce sub-optimal separations. **Recommendation:** test first your cells' preferred culture medium in the separation protocol. If this proves sub-optimal, use **Dead-Cert® Yield-Enhancing Separation (YES) Medium (DC-004)** in the separation steps and then return the separated cells to their regular medium at the end of the procedure.

2. Cell Clumping

The key to efficient separation using magnetic particles is that cells remain in mono-dispersed suspension during the separation protocol. Clumping of non-viable with viable cells can profoundly reduce separation efficiencies and yields. **Recommendation:** Use culture media containing low levels of Ca^{2+} and Mg^{2+} which can otherwise promote cell clumping. Choosing a low temperature can also help. Alternatively use **Dead-Cert® YES Medium (DC-004)** in the separation steps as this has been specifically designed to reduce cell clumping.

3. Non-Viable Cell Numbers

The generic separation protocol supplied with **Dead-Cert® Nanoparticles** is designed to work optimally with an input cell population of 1 to 5×10^6 cells containing approximately 50% non-viable cells. Note that simple viability assessments with vital dyes such as trypan blue and propidium iodide can belie the true viability of a cell population: apoptotic cells with intact membranes remain unstained with these dyes but will be recognised by **Dead-Cert® Nanoparticles**. High percentages of non-viable cells (both apoptotic and dead) could warrant increasing the ratio of **Dead-Cert® Nanoparticles** to cells. **Recommendation:** Be sure that viability is assessed accurately in input and output populations. Vital dyes can be sufficient in many cases but use of apoptosis detection agents such as **Dead-Cert® imab6 (DC-006)** may be required. Increase (or decrease) nanoparticle numbers and/or extend (or reduce) incubation of cells with nanoparticles to suit the levels of non-viable cells in the input population. A second round of separation can improve results when high proportions of non-viable cells are present.

4. Physical Parameters: Scale-up

Changing the incubation temperature and/or the physical dimensions of the separation vessel can provide improvements in performance of **Dead-Cert® Nanoparticles**, particularly for larger-scale separations. For example, raising the temperature of incubation of cells with nanoparticles can reduce the incubation time and can help with scaled-up separations of large numbers of cells. Cells that phagocytose particles at physiological temperature should, however, be separated in the cold in order to avoid low yields of viable cells. When dealing with large numbers of cells, the incubation and separation volumes, as well as the particle number (ie volume of particle stock used) should be scaled up accordingly. Periodic gentle agitation of the tube during the incubation step can also provide improvement. **Recommendation:** Using the generic protocol supplied with the product as a guide, increase the incubation volume, tube size and separation volume. For example, to scale up to 20 to 50×10^6 cells, use $\sim 100 \mu\text{l}$ **Dead-Cert® Nanoparticles**, incubate in ~ 1 ml cell culture medium or **Dead-Cert® YES Medium** in a 15 ml polypropylene tube at the temperature of choice with occasional gentle agitation. At the end of the incubation step, add 4 to 9 ml of cell culture medium or **Dead-Cert® YES Medium** and separate using a magnet suitable for 15 ml tubes (these can be purchased from a number of suppliers) following the same overall procedures as set out in the generic protocol, except that a separation time of 5-10 minutes on the magnet is recommended.

A list of frequently-asked questions about **Dead-Cert® Nanoparticles** is available at www.immunosolv.com