

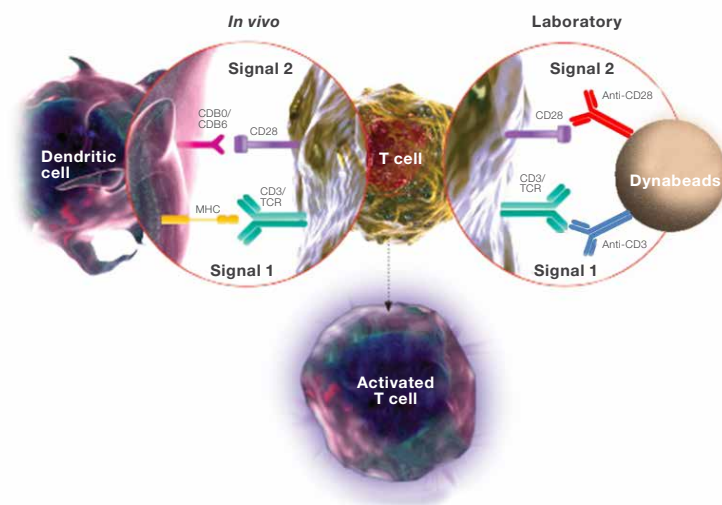
# Cell culture quality control during T cell expansion

## Introduction

There is great interest in immuno-oncology research as more biotherapeutics have shown success in clinical trials. These new drugs take many forms, including chimeric antigen receptor (CAR) T cells, checkpoint blockade antibodies, and T cell-engaging bispecific antibodies. All of these immunotherapy strategies improve the ability of T lymphocytes to target and kill cancer cells.

Research and development of new immunotherapies requires robust techniques to purify and culture T cells. Here we describe methods for isolation of T cells from blood followed by activation and expansion using Invitrogen™ Dynabeads™ magnetic beads without the need for feeder cells or antigens. Overstimulation of T cells can lead to activation-induced cell death, and variations in cell culture conditions or technique can affect T cell viability.

Obtaining maximum viability is critical to the optimal performance of T cells in functional assays. It is important to be able to quickly assess the health of T cells using a small sample of cells. The Invitrogen™ Countess™ 3 FL Automated Cell Counter provides fast, accurate measurements of cell viability and concentration that can be performed both before and after isolation and activation, saving time and precious cells for future experiments. Additionally, the Invitrogen™ EVOS™ M3000 Imaging System can be used to monitor the expansion of T cells without disturbing the activation process or requiring extended time outside of the incubator.



## Materials\*

- EVOS M3000 Imaging System (Cat. No. AMF3000)
- Countess 3 FL Automated Cell Counter (Cat. No. AMQAF2000)
- Invitrogen™ Countess™ Cell Counting Chamber Slides (Cat. No. C10228)
- Invitrogen™ Trypan Blue Stain (0.4%) (Cat. No. T10282)
- Gibco™ CTS™ OpTmizer™ T Cell Expansion SFM (Cat. No. A1048501)
- Gibco™ PBS, pH 7.4 (Cat. No. 10010023)
- Invitrogen™ Dynabeads™ Untouched™ Human T Cells Kit (Cat. No. 11344D)
- Gibco™ Dynabeads™ Human T-Activator CD3/CD28 (Cat. No. 11131D)
- Invitrogen™ DynaMag™-15 Magnet (Cat. No. 12301D)

\*Intended use of the products mentioned on this page vary. For specific intended use statements please refer to the product label.

## Methods

### Isolation of T cells from whole blood

Human peripheral blood was mixed with an equal volume of PBS (20 mL each) and carefully layered over 15 mL of Ficoll™ solution and centrifuged at 400 x *g* for 30 minutes with minimal acceleration and deceleration. The peripheral blood mononuclear cell (PBMC) layer was isolated and washed with PBS. The cells were mixed with an equal volume of trypan blue stain (10  $\mu$ L each), and 10  $\mu$ L of the mixture was pipetted into the chambers of a Countess slide (Figure 1). The Countess 3 FL Automated Cell Counter was used to determine cell viability and concentration. Cell concentration was then adjusted with PBS to  $1 \times 10^6$  cells/mL.

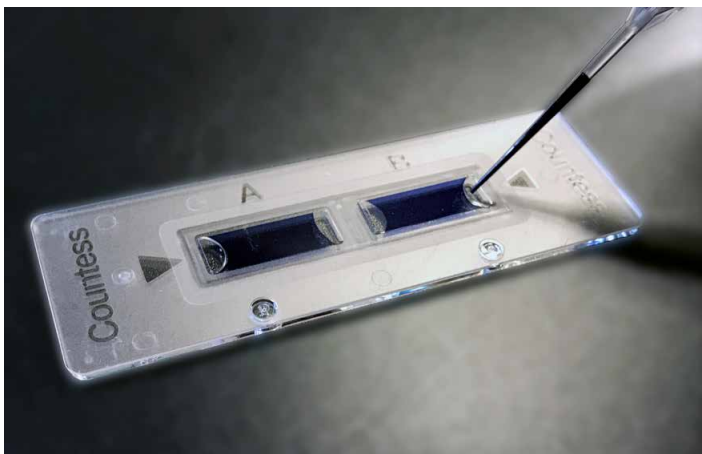


Figure 1. Loading a Countess chamber slide.

Negative isolation of T cells was performed using the Dynabeads Untouched Human T Cells Kit as described in the product insert. Briefly, the provided antibody mixture and Depletion Dynabeads magnetic beads were used to remove B cells, natural killer (NK) cells, monocytes, platelets, dendritic cells, granulocytes, and erythrocytes, leaving T cells for further use.

### Activation of human T cells

Isolated T cells were analyzed on the Countess 3 FL Automated Cell Counter to determine cell viability and concentration. Cell concentration was adjusted to  $1 \times 10^6$  cells/mL in CTS OpTmizer T Cell Expansion SFM. Dynabeads Human T-Activator CD3/CD28 was added at a bead:cell ratio of 1:1. Cells were plated in a T-75 flask in a cell culture incubator at 37°C with 5% CO<sub>2</sub>.

### Monitoring of T cell proliferation and viability

Every 24 hours, the flask was removed from the incubator and viewed at 40x magnification on an EVOS M3000 Imaging System. Initially the T cells and beads were observed to settle to the bottom of the flask. After 24 hours, the cells and beads began to bind and form clumps that grew in size as the cells proliferated. The cell clumps were weakly attached and could easily be disrupted by pipetting the medium up and down after 5 days of expansion. Using normal cell counting techniques it may be difficult to obtain an accurate measure of cell concentration when the beads are still in the culture flask. The Countess software allows cells to be gated based on size, brightness, and circularity, allowing easy inclusion or exclusion of debris or specific cell populations. Increasing the lower size threshold sets the instrument to only count the larger T cells and not the smaller beads.

Once the desired expansion was completed, the beads and cells were resuspended in medium and transferred to a 15 mL conical tube. The tube was then placed in a DynaMag-15 Magnet for 2 minutes. The beads bound to the side of the tube while purified, activated T cells were removed along with the medium and could be used for future experiments.

## Results

The Dynabeads Untouched Human T Cells Kit was used to prepare isolated T cells that were 95% viable as determined by the Countess 3 FL Automated Cell Counter (Figure 2).

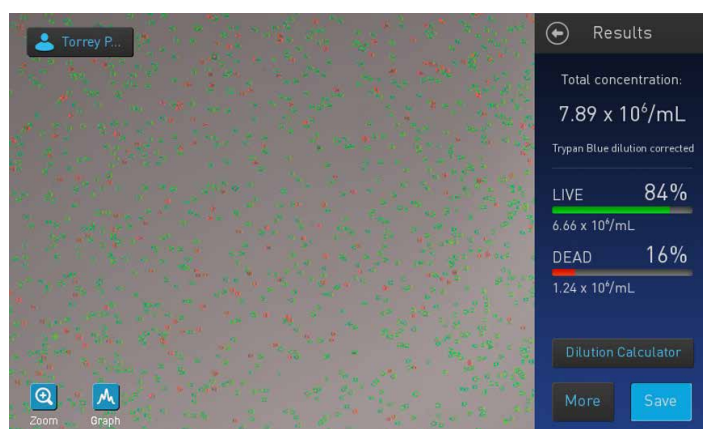
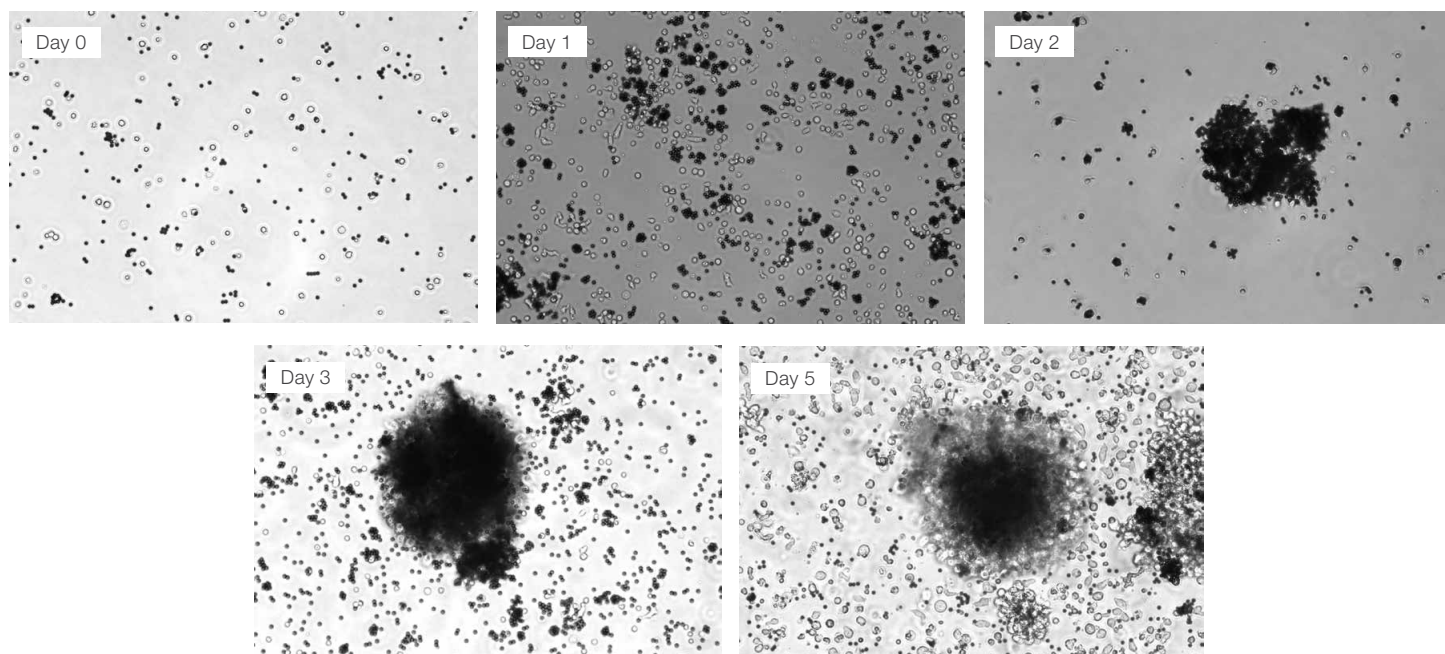


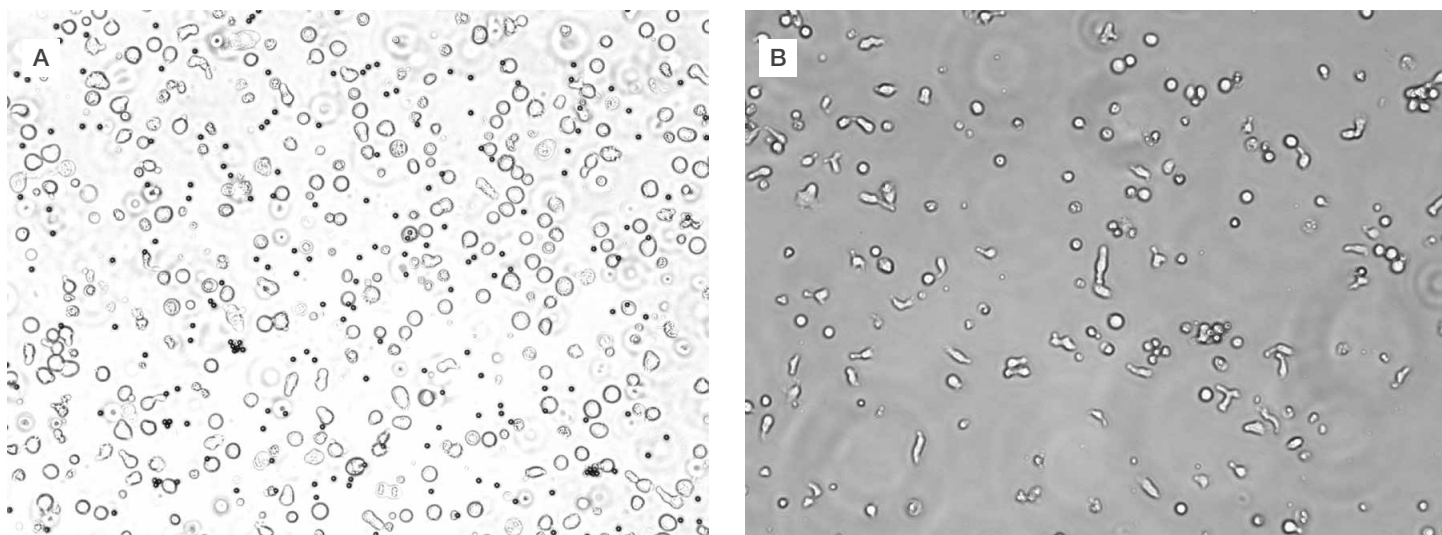
Figure 2. Viability and concentration of isolated T cells determined using the Countess 3 FL Automated Cell Counter. T cells isolated with the Dynabeads Untouched Human T Cells Kit were 95% viable.

These isolated T cells were then activated and expanded with Dynabeads Human T-Activator CD3/CD28. This process could be easily monitored by viewing the flask of T cells and beads on the

EVOS M3000 Imaging System without disturbing the cells or the activation process (Figures 3 and 4).

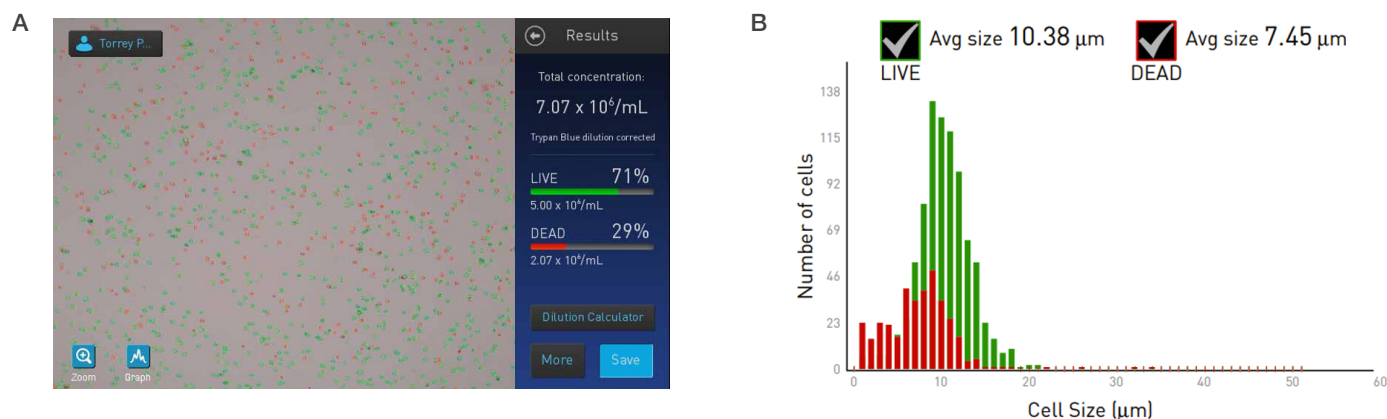


**Figure 3. Expansion of T cells with Dynabeads Human T-Activator CD3/CD28.** Every 24 hours, the flask containing isolated T cells and Dynabeads Human T-Activator CD3/CD28 was viewed on the EVOS M3000 Imaging System. Clumping of the beads was observed 1 day after activation, and the clumps increased in size as the T cells became activated and proliferated.



**Figure 4. Isolation of activated T cells.** (A) Image was taken immediately after cells were dispersed from clumps into a single-cell suspension with beads still present. (B) Image was taken after magnetic removal of beads when only activated T cells remained.

Viability and concentration of the activated T cells were measured on the Countess 3 FL Automated Cell Counter. Beads were excluded from the cell count by increasing the size threshold using the gating feature of the instrument software. Viability of the activated T cells could be determined even in the presence of beads (Figure 5).



**Figure 5. Determination of cell viability and concentration in the presence of Dynabeads magnetic beads.** (A) The Countess 3 FL Automated Cell Counter can determine cell concentration and viability even with beads present. (B) The size threshold on the software can be adjusted to exclude beads (faded colored bars) while still providing accurate measurements of cell viability and concentration.

## Conclusion

This workflow enables the isolation, activation, and expansion of robust T cells for downstream immuno-oncology applications, a critical need in this research area for cancer treatment and therapeutics.

The Countess 3 FL Automated Cell Counter provides fast, accurate measurements of T cell viability and concentration both after initial isolation and activation, even when Dynabeads Human T-Activator CD3/CD28 is present. These critical in-process measurements help determine if the cells are healthy and should continue to be expanded. Dynabeads Human T-Activator CD3/

CD28 provides strong activation of T cells grown in CTS OpTmizer T Cell Expansion SFM. The EVOS M3000 Imaging System enables a quick check of T cell proliferation (indicated by cell clumping) without disruption of the cells or extended time outside the incubator. The combination of these reagents with the ability to monitor T cell activation, proliferation, and viability with minimal cell disturbance enables production of high-quality T cells that can be used in immuno-oncology experiments to help accelerate knowledge in this important area of cancer therapeutics.

## Ordering information

Description	Quantity	Cat. No
EVOS M3000 Imaging System	1 instrument	AMF3000
Countess 3 FL Automated Cell Counter	1 instrument	AMQAF2000
Countess Cell Counting Chamber Slides	50 slides	C10228
Trypan Blue Stain (0.4%)	2 x 1 mL	T10282
CTS OpTmizer T Cell Expansion SFM	1,000 mL	A1048501
PBS, pH 7.4	500 mL	10010023
Dynabeads Untouched Human T Cells Kit	1 kit	11344D
Dynabeads Human T-Activator CD3/CD28	2 mL	11131D
DynaMag-15 Magnet	1 each	12301D

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