Cell therapy

Quantification of residual magnetic beads

Introduction

This protocol is used to manually quantify the number of magnetic beads from Gibco™ CTS™ Dynabeads™ and CTS™ Detachable Dynabeads™ products when used in suspension with or without cells, using an inverted light microscope. It can be used to count residual beads in expanded cells after magnetic removal of CTS Dynabeads magnetic beads.

After harvesting a representative sample containing CTS Dynabeads magnetic beads (e.g., from debeaded cells), the sample is centrifuged and the supernatant is removed without disturbing the pellet containing cells and beads. To accurately count the beads in the sample, it is critical to lyse the cells (if present) in the sample, as this will remove the interference from cells and cell debris during analysis. Deoxyribonuclease I (DNase I) and proteinase K are added to the sample in separate steps with heat incubation to lyse the cells. The sample is centrifuged and the supernatant is discarded. The pellet is resuspended in a known volume, before the sample is counted in a hemocytometer and the concentration of beads in the initial sample is calculated. The sample is loaded onto a Kova™ Glasstic™ Slide 10 with Grids. The Kova Glasstic Slide 10 with Grids has a grid system that allows quantification based on counted beads. By counting a representative number of squares on the light microscope, the number of beads in the sample can be calculated based on the known volume in each square, following instructions from the Kova slide manual. The following equations can be used to calculate the number of beads in the starting sample.

Equation 1 gives the absolute number of beads in the starting sample; if you did not start out with 3 million cells, use equation 2 to calculate the number of beads per 3 million cells. The general workflow for this method can be seen in Figure 1.

Equation 1

$$\text{Number of beads in starting sample} = \frac{n_{\text{beads counted}}}{n_{\text{squares analyzed}} \times 0.01089\ \mu L/\text{square}} \times V_{\text{final volume of sample}}$$

where $n_{\text{beads counted}}$ is the total number of beads counted under the light microscope; $n_{\text{squares analyzed}}$ is the number of squares counted (typically 81 squares); the constant (0.010189 µL/square) is the volume of each square (dimensions are 0.1 x 0.33 x 0.33 mm^3); and $V_{\text{final volume of sample}}$ is the volume that the sample was resuspended in (suggested volume 12 µL).

Equation 2

$$\text{Number of beads per 3 million cells} = \frac{\text{Number of beads in starting sample} \times 3 \times 10^6}{\text{Number of cells in starting sample}}$$

where “Number of beads in starting sample” is the result of equation 1 and “Number of cells in starting sample” is the absolute number of cells in the starting sample (typically 3 million cells).

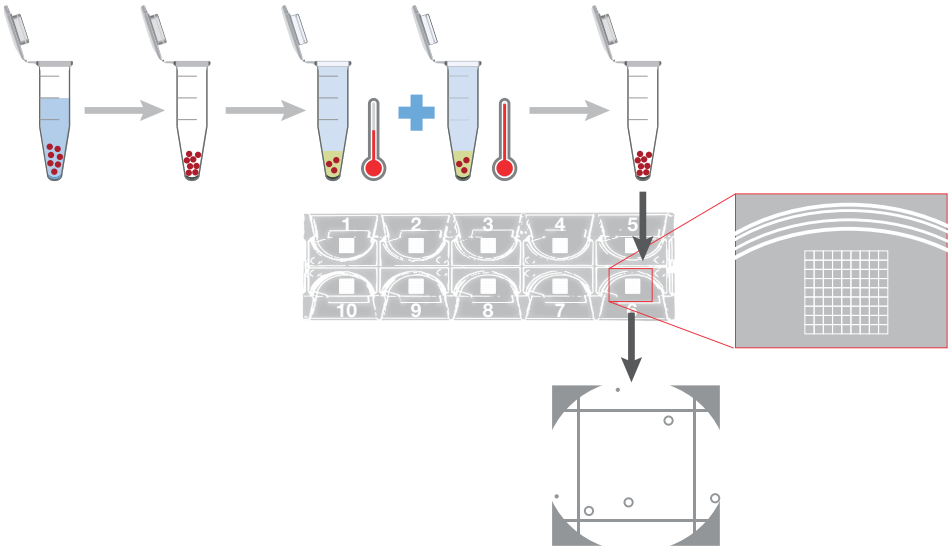

Figure 1. Workflow of sample preparation and counting of CTS Dynabeads magnetic beads on Kova Glasstic slides using an inverted light microscope. The sample is centrifuged and the supernatant is removed. Buffer with DNase I is added and incubated at 37°C for 20 min. Proteinase K is added to the buffer and the sample is further incubated at 65°C for 20 min. The sample is centrifuged and the resultant pellet is resuspended in a small volume of buffer. A portion is loaded onto a chamber of a Kova Glasstic slide, and beads are counted in a selected number of squares in the grid using an inverted light microscope.

Materials

The reagents and equipment required for this protocol are listed in Table 1 and Table 2.

Table 1. Reagents required for this protocol.

Reagent	Vendor	Cat. No.
RIPA Lysis and Extraction Buffer	Thermo Fisher Scientific	89901
DNase I	Thermo Fisher Scientific	18047-019
Proteinase K (20 mg/mL)	Thermo Fisher Scientific	25530-049

Table 2. Equipment required for this protocol.

Equipment	Vendor	Comment
Kova Glasstic Slide 10 with Grids (Cat. No. 87144)	Kova	Single-use counting slide with 10 chambers
Zeiss™ Axiovert™ 25 Inverted Phase Contrast Microscope	Zeiss	Or equivalent
Microcentrifuge 5415 R	Eppendorf	Or equivalent
Julabo™ ED-5M Open Bath Circulator	Julabo	Or equivalent; used for heating at 37°C and 65°C
Thermo Scientific™ LP Vortex Mixer	Thermo Fisher Scientific	Or equivalent
Galaxy Mini Centrifuge	VWR	Or equivalent
Pipettes for various volume ranges	Various	–
Magnet for tubes (e.g., Gibco™ CTS™ DynaMag™ Magnet for 1–2 mL Eppendorf tubes)	Thermo Fisher Scientific	Size of magnet must fit centrifuge tube with harvested sample
Conical centrifuge tubes	Various	Single-use tubes for harvesting test samples; the size of the tube must fit the volume of harvested sample
Manual differential counter	Various	–

Protocol

1. In a conical centrifuge tube, harvest 3 million cells from the sample containing the unknown number of CTS Dynabeads magnetic beads.
2. Centrifuge the harvested sample at maximum speed (~16,100 x *g*) in a tabletop centrifuge for 5 minutes at room temperature.* Note the orientation of the tube inside the centrifuge to keep track of where the pellet (containing beads and cells) is collected inside the tube.
3. Place the tube on a magnet, with the pellet facing the magnet. Leave the tube on the magnet for 1 minute.
4. Use a pipette to remove the supernatant without disturbing the pellet. During pipetting, place the tip on the tube wall opposite to where the pellet is collected.
5. Remove the tube from the magnet, add 1 mL of RIPA buffer, and resuspend the pellet without generating bubbles.
6. Add 10 µL of DNase I and carefully resuspend using a P-1000 pipette.
7. Incubate the tube at 37°C for 20 minutes.
8. Add 50 µL of proteinase K and carefully resuspend using a P-1000 pipette.
9. Incubate the tube at 65°C for 20 minutes.
10. Centrifuge the tube at maximum speed (~16,100 x *g*) in a tabletop centrifuge for 5 minutes at room temperature.* Note the orientation of the tube inside the centrifuge to keep track of where the pellet (containing beads) is collected inside the tube.
11. Place the tube on a magnet, with the pellet facing the magnet, for 1 minute.
12. Remove the supernatant without disturbing the pellet. During pipetting, place the pipette tip on the tube wall opposite to where the pellet is collected.
13. Remove the tube from the magnet, vortex briefly, and centrifuge the tube using a mini centrifuge or a tabletop centrifuge to collect all liquid at the bottom of the tube.
14. Measure the volume in the tube and add RIPA buffer to a total volume of 12 µL. If the tube contains more than 12 µL, add RIPA buffer to a total volume of 24 µL. Record which samples were resuspended in which volume. Note: For precise measurement of sample volume, use a 2–20 µL pipette tip (e.g., Eppendorf™ Dualfilter T.I.P.S™ 2–20 µL pipette tips, Cat. No. 0030078535).
15. Vortex the tube briefly.
16. Load the sample onto a Kova Glasstic slide chamber such that the sample covers the grid, all 81 squares. For samples that were adjusted to 24 µL in step 14, split the sample into two chambers for counting and combine the results. Note that the liquid can evaporate from the slide, so we do not recommend loading many samples at a time. If there are air bubbles covering parts of the grid, do not count the affected squares nor the adjacent squares.
17. Determine the approximate number of beads per square in the chamber using an inverted light microscope.**
18. Determine the number of beads in each sample and numbers of beads per 3 million cells using equations 1 and 2, respectively.

* For samples with high cell concentrations, harvest samples in 1.5–2 mL microcentrifuge tubes and use maximum speed during centrifugation (~16,100 x *g*). For samples with lower concentrations, use 15 mL or 50 mL conical tubes and centrifuge at maximum speed.

** Light microscope settings: The optimal setting is to use a 20–40x ocular for counting magnetic beads. Inspect each grid at a range of focal planes. The beads will appear brown and uniform in size. If using a 10x focal plane, the beads will appear black and white, depending on the focal plane.

General guidelines for counting CTS Dynabeads magnetic beads using a Kova Glasstic slide

- Count beads within the boundaries of the squares. Beads that are located on the grid lines will not be counted, as they are not part of the area that is used to calculate the volume of a square.
- Never count fewer than 3 squares per sample.
- If the bead counting indicates less than 1 bead per square, on average, always count all 81 squares.
- If the sample is highly concentrated, containing >15 beads per square, dilute the sample and then load it into a new chamber on the Kova Glasstic slide. Multiply the result by the dilution factor to obtain the final concentration.
- See Figure 2 for procedures for counting squares depending on the number of squares to count.
- Always include positive and negative sample controls for reference when counting. To be able to distinguish actual beads from remaining cell debris during the counting procedure, a positive control can be RIPA buffer spiked with a known amount of CTS Dynabeads magnetic beads, and a negative control can be lysed cells without CTS Dynabeads magnetic beads.
- Use a manual differential counter to more easily keep track of the number of counted beads within each square.
- Limit of detection (LOD) and limit of quantification (LOQ) is one counted bead in 81 squares, which corresponds to 13.6 beads/3 million cells when starting with 3 million cells and the sample is resuspended in 12 µL on a Kova Glasstic slide.

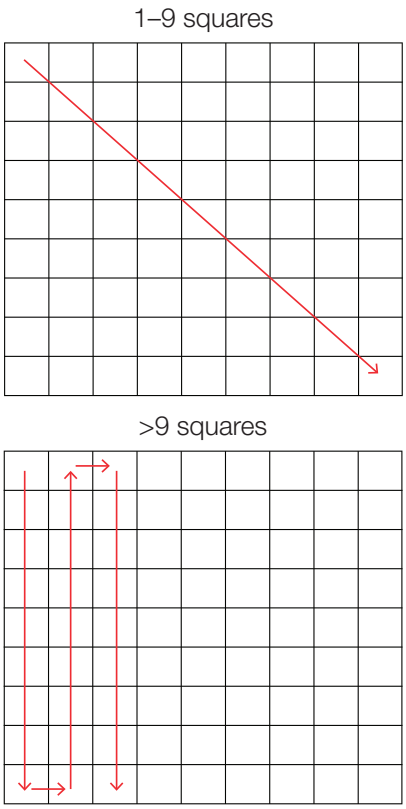

Figure 2. Best practice for counting squares of a Kova Glasstic slide chamber. If counting ≤9 squares, follow the diagonal from the top left to the bottom right. If counting >9 squares, start from the top left and go vertically down the column, then shifting right to the next column and moving up vertically. Repeat this procedure until the intended number of squares are counted.

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