anti-Human CD3/CD28 T Cell Activation Beads

Catalogue No.: K132

Size: 1mL/1ml*5

Endotoxin：< 0.5 EU/mL

Storage:

2-8°C for 12 months, freezing must be avoided.

Principle of the Assay:

The activation and proliferation of T cells in the body usually require two signals. One type is the specific antigen stimulation signal generated by the binding of TCR/CD3 to the specific MHC II antigen peptide complex on the surface of antigen-presenting cells, and the other is the non-specific co-stimulatory signal, with CD28 being one of the co-stimulatory molecules. The anti-HumanCD3/CD28 T Cell Activation Beads have both human CD3 and CD28 monoclonal antibodies conjugated on their surface, which can provide the major and co-stimulatory signals required for T cell activation and expansion, thereby inducing T cell activation and proliferation.
The magnetic beads used in this product have a diameter of 4.5 μm and are non-porous, monodisperse superparamagnetic microbeads. The beads possess superparamagnetism and can be uniformly dispersed in solution. When placed in a magnetic field (such as a magnetic stand), the beads respond rapidly and are adsorbed onto the tube wall, facilitating efficient washing or removal; when the magnetic field is removed, the beads have no residual magnetism, avoiding cell damage.
The magnetic bead density of this product is 5×10^7 beads/mL, and 1 mL can be used for the expansion of 5×10^7 PBMCs or T cells. The storage system is a phosphate buffer (pH 7.4) containing 0.1% HSA.

Additional Materials Required:

RPMI-1640 complete medium, Human IL-2 protein

Cell culture plate

Magnetic rack, Vortex mixer, CO2 cell incubator

Assay Procedure (For reference):

The following operations must be carried out under aseptic conditions.

- **Washing of Anti-Human CD3/CD28 T Cell Activation Beads**
  a) Before use, vortex the beads for 30 seconds to obtain a uniform suspension;
  b) According to the experimental requirements, transfer the required amount of bead suspension to a 1.5 mL centrifuge tube;
  c) Place the centrifuge tube on a 1.5 mL magnetic rack (not provided) for 3 minutes, and remove the supernatant with a pipette (the tube should be placed on the magnetic rack at this time);
  d) Add 1 mL of PBS (pH 7.4), vortex for 10 seconds to mix well;
  e) Place the centrifuge tube on a 1.5 mL magnetic rack (not provided) for 3 minutes, and remove the supernatant with a pipette (the tube should be placed on the magnetic rack at this time);

f) Add an equal volume of RPMI-1640 complete medium to the bead suspension, vortex for 10 seconds to resuspend the beads, and set aside for use.

- **T Cell Activation**

  a) Seed cells at a concentration of 1×10^6 cells/mL in an appropriate cell culture plate;
  b) Add 20 µL of beads for every 1×10^6 cells, that is, the ratio of beads to T cells is 1:1, and mix well by pipetting;
  c) Culture in a 37°C CO2 cell incubator;
  d) Collect activated T cells according to the experimental requirements;
  e) For flow cytometry applications, remove the beads, transfer the cells to a centrifuge tube, place it on a magnetic rack for 3 minutes, and collect the supernatant containing the cells for detection.

- **T Cell Expansion**

  a) Seed cells at a concentration of 1×10^6 cells/mL in an appropriate cell culture plate;
  b) Add 20 µL of beads for every 1×10^6 cells, that is, the ratio of beads to T cells is 1:1, and mix well by pipetting;
  c) Add Recombinant Human IL-2 (Cat. No: Pr20697, recommended concentration 100 ng/mL) to the culture medium;
  d) Culture in a 37°C CO2 cell incubator;
  e) Count the cells every two days. When the cell density exceeds 2.5×10^6 cells/mL or when the medium turns yellow, perform cell passage to maintain the cell density at 0.5-1×10^6 cells/mL and supplement with human IL-2 protein.

Notes:

1. This product is for scientific research use by professionals only.
2. Please pay attention to safety precautions and follow the laboratory reagent operation norms. For your safety and health, please wear a lab coat and disposable gloves during the operation.
3. Before using magnetic beads, they should be thoroughly shaken and mixed. During the aspiration process, avoid air bubbles to prevent differences in the amount of magnetic beads from affecting subsequent experimental results.
4. Place the centrifuge tube on the magnetic rack for no less than 3 minutes to avoid loss of magnetic beads. When removing the supernatant after magnetic separation, do so carefully and slowly to prevent loss of magnetic beads.
5. When the volume of magnetic beads is less than 0.1 mL, it is recommended to use 0.1 mL for washing. When the volume is greater than 0.1 mL, use an equal volume for washing.
6. After magnetic separation and removal of the supernatant, resuspend the magnetic beads in liquid as soon as possible to prevent drying, which may affect the usage effect.
7. If the cell activation effect is poor, you can appropriately increase the amount of magnetic beads. If a large number of cells die, you can appropriately reduce the amount of magnetic beads.
8. Before analyzing with a flow cytometer, remove the magnetic beads.