Bio-ReCell®

Atract™ T-Cell Activation Kit V2, CD2/CD3/CD28

For activation of human T cells | Code: UM-ATV2_v4

Kit Format	4 × 10 mg (4 × 200 µL) Atract™ V2, CD2/CD3/CD28; cell culture grade	4 × 50 mg (4 × 1 mL) Atract™ V2, CD2/CD3/CD28; cell culture grade
Catalogue Number	AT-V2-010	AT-V2-050
Capacity	Sufficient to activate up to 24 × 10^6 peripheral blood mononuclear cells (PBMCs).	Sufficient to activate up to 120 × 10^6 peripheral blood mononuclear cells (PBMCs).
Characteristics	The Atract™ Kit V2 is biocompatible and non-toxic. All components are supplied in azide-free buffer.	
Storage	Transport at ambient temperature. Upon receipt, store at 2–8 °C, protected from light. Do not freeze. The expiration date is indicated on the box and on the vial.	

1. Principles of Atract™ T-Cell Activation Kit V2

- Designed to activate and expand human T cells.
- Comprises biocompatible composites with irreversibly bound anti-CD2, anti-CD3 and anti-CD28 antibodies (Atract™ V2 reagent).
- Designed to mimic the role of antigen-presenting cells and activate naïve T cells derived from PBMCs.
- T cells, activated with Atract™ V2 reagent, expand through cultivation in a medium, supplemented with interleukin 2 (IL-2) for up to 14 days.

2. Materials Required

- Activation/expansion medium: basal T-cell medium supplemented with 10% FBS or 5% serum replacement and 200 U/mL of IL-2 or a T-cell medium prepared according to your protocol.
  Optional: addition of antibiotics.
- Tissue culture plate (treated) or G-Rex® platform.
- 20-30 µm strainers, suitable for filtering the suspension.

3. Protocol

The Atract™ V2 reagent sediments quickly. Always mix the reagent suspensions by pipetting right before transferring. Use the same pipette tip for mixing and transferring the suspension.

All steps in the protocol must be performed under sterile conditions.

3.1. Atract™ V2 Reagent Preparation and Cell Activation

This protocol describes how to set up one well of a 96-well plate. If using a different format, refer to **Table 1** or **Table 2** for the recommended quantities of Atract™ V2 reagent and number of cells.

Note: *Due to quick sedimentation of Atract™ V2 reagent, additional precautionary steps are needed for 96-, 48-, 24-, and 12-well plate protocols. When mixing and transferring, the pipetting volume should be at least one-fifth of the total volume of the Atract™ V2 reagent in the tube. Therefore, to ensure accurately prepared aliquots of Atract™ V2 reagent, intermediate aliquots are needed.*

Note: *If using Atract™ Kit V2, 10 mg/200 µL (Cat. No.: AT-V2-010), start with step 3.*

1. Thoroughly mix the Atract™ V2 reagent using a 1 mL pipette.
2. Immediately after mixing, transfer 10 mg (200 µL) of the Atract™ V2 reagent into a fresh microcentrifuge tube.
   Note: this volume equals to one-fifth of the total reagent volume in the vial.
3. Thoroughly mix the Atract™ V2 reagent using a 200 µL pipette.
4. Immediately after mixing, transfer 2.5 mg (50 µL) of the Atract™ V2 reagent into a fresh microcentrifuge tube.
5. Thoroughly mix the Atract™ V2 reagent using a 10 µL pipette.
   Optional: To wash the beads, add 1 mL of the activation/expansion medium and short spin the tube to sediment the Atract™ V2 reagent. Discard the supernatant and resuspend the Atract™ V2 reagent in 50 µL of fresh medium.
6. Immediately after mixing, transfer 0.5 mg (10 µL) of the Atract™ V2 reagent into a 96-well plate.
7. Add 0.3 × 10^6 cells into the well, containing Atract™ V2 reagent, to reach desired working volume.
8. Incubate at 37 °C and 5% CO_2 for up to 2 days.

Note: *A uniform distribution of Atract™ V2 reagent is needed for optimal activation. To achieve this in larger plate formats, combine the cells with the reagent aliquot, gently mix and pipette the mixture evenly into the well (e.g., release the cell/reagent mixture at three points, spaced evenly apart). The culture plate should be steadily placed in the incubator.*

Table 1: Recommended Atract™ V2 reagent quantities and number of cells for the desired format.

	96-well plate	24-well plate	12-well plate
Atract™ V2 reagent/well (mg)	0.5 (10 µL)	2.1 (42 µL)	6 (120 µL)
Cells/well (× 10^6)	0.3	1	3

Note: *Recommended values are based on Bio-ReCell's in-house tests. For non-specified surfaces, we recommend using 1.5 mg of Atract™ V2 reagent per cm^2 of the activation surface and 0.6 × 10^6 cells per mg of Atract™ V2 reagent. Ratios can be further optimized based on your specific experimental designs.*

Note: *Use the standard working volumes for each platform where Atract™ V2 reagent is used or according to your usual laboratory practice.*

3.2. Expansion in Well Plates

1. After 48 h, gently pipette the suspension up and down to homogenize the cell culture.
   Optional: The Atract™ V2 reagent can be removed on day 2 using 20-30 µm strainers.
2. Transfer the cell suspension to a new well for expansion at a concentration of 0.5 × 10^6 cells/mL and maintain the concentration around this value throughout the expansion process.
   Optional: The suspension can also be transferred to a larger well, but the volumes must be adjusted accordingly.
3. Split the cell suspension daily and add fresh activation/expansion medium, supplemented with 200 U/mL of IL-2.
4. Incubate at 37 °C and 5% CO_2 for 7 days or according to your protocol.
5. Mix to resuspend the cells in the well.
6. Filter suspension through a 20-30 µm strainer to remove Atract™ V2 reagent.
7. Collect the filtrate containing expanded T cells.

3.3. G-Rex® Platform Activation and Expansion Guide

Table 2: Atract™ V2 reagent quantities and number of cells for the desired protocols.

	G-Rex® 6M Well Plate	G-Rex® 50M	G-Rex® 100M
Atract™ V2 reagent/well (mg)	16 (320 µL)	80 (1600 µL)	160 (3200 µL)
Cells/well (× 10^6)	5	25	50
Starting volume on day 0/well (mL)	5	25	50
Expansion volume on day 2-8/well (mL)	100	500	1000

Harvesting volume on day 9/well (mL)	20	100	200

3.4. Activation and Expansion in G-Rex® 6M Well Plate

1. Thoroughly mix the Atract™ V2 reagent using a 1 mL pipette.
2. Immediately after mixing, transfer 16 mg (320 µL) of the Atract™ V2 reagent into a fresh tube.
3. Combine 5 × 10^6 cells with the Atract™ V2 reagent and transfer to a G-Rex® 6M Well Plate. If necessary, add more activation/expansion medium to reach the starting volume.
4. Incubate at 37 °C and 5% CO_2.
5. After 48 h, add 95 mL of the activation/expansion medium to each well to reach the expansion volume.
6. Incubate at 37 °C and 5% CO_2 for 7 days or according to your protocol.
7. On the harvest day, remove 80 mL of the medium to reach the harvesting volume. To not disrupt the sedimented cells and reagent, carefully remove medium from the top.
   Note: Removal of 80% of medium is optional and is performed to concentrate the cells.
8. Swirl the plate gently for 30 seconds to resuspend the cells.
9. Filter suspension through a 20-30 µm strainer to remove Atract™ V2 reagent.
10. Collect the filtrate containing expanded T cells.

***Note**: For other G-Rex® platform sizes, modify the volumes according to **Table 2**.*

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