Assessing T Cell Activation & Suppression in Drug Development

T Cell Suppression

Regulatory T (Treg) cells suppress exuberant immune activation and promote immunological tolerance. The most important subset of Treg cells expresses the transcription factor forkhead box P3 (FOXP3). Treg cells modulate the immune response in numerous settings, including autoimmune disease, allergy, microbial infection, tumour immunity, organ transplantation, fetal–maternal tolerance and even obesity.[1] Manipulating Treg functions may be an important immunomodulatory tool in the regulation of innate and adaptive immunity.

Assessing Pre-Clinical Compounds in In Vitro Treg Suppression Assays

In vitro suppression assays are used to determine the suppressive capacity of Treg cells by measuring their abilities to suppress the proliferation of responding $CD4^+$ or $CD8^+$ T cells. In a suppression assay, human Treg cells are purified from a leukapheresis sample. The responder cells from the same leukapheresis sample are labeled with proliferation tracking dye. The responder cells are incubated with different ratios of Treg cells and stimulated for proliferation in the presence or absence of immunomodulatory drug. The proliferation of the responder cells in each treatment condition are tracked by flow cytometry after 4 - 5 days of activation.

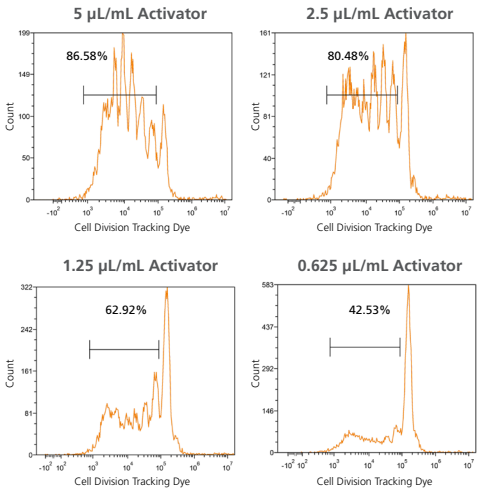

Figure 1. Stimulation of Peripheral Blood Mononuclear Cells with ImmunoCult™ Human CD3/CD28 T Cell Activator

Peripheral blood mononuclear cells (PBMCs) were stimulated with different concentrations of ImmunoCult™ Human CD3/CD28 T Cell Activator (Catalog #10971) in ImmunoCult™-XF T Cell Expansion Medium (Catalog #10981) for 5 days. Proliferation of PBMCs was measured by flow cytometric analysis.

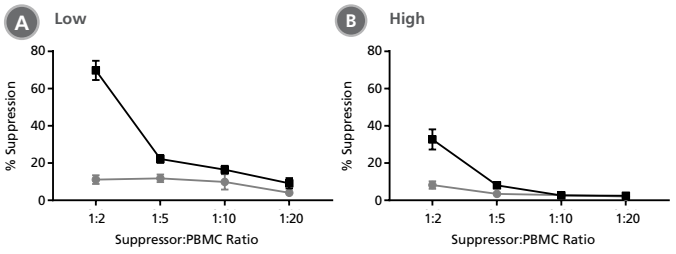

Figure 2. Treg Cells Suppress $CD8^+$ T Cell Proliferation

PBMCs were stimulated with different concentrations of ImmunoCult™ Human T Cell Activator and cultured in the presence of various ratios of Treg cells for 4 days followed by flow cytometric analysis. The suppression response at the 1:2 Treg:PBMC ratios were 70% and 33% when stimulated with (A) low and (B) high concentrations of ImmunoCult™ Human T Cell Activator, respectively. Black lines indicate $CD4^+CD25^+$ Treg cells and grey lines indicate $CD4^+CD25^-$ T cells (mean ± SD, n = 3, single donor).

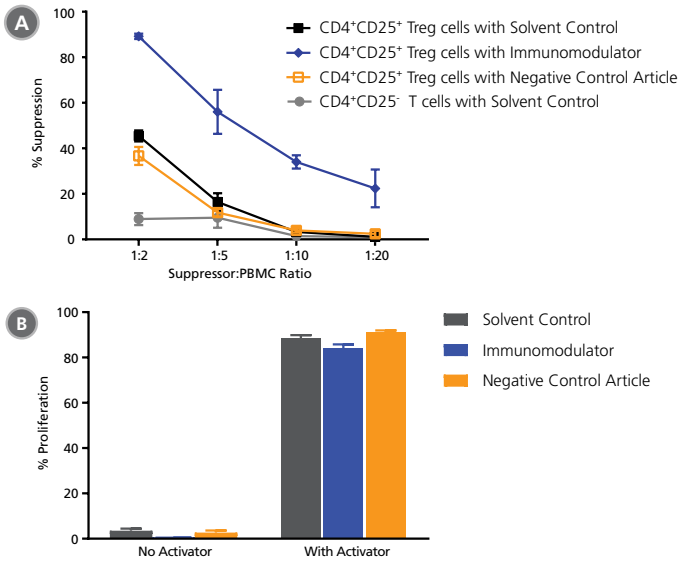

Figure 3. Immunomodulatory Test Compound Enhances Treg Cell Suppression Function but Has No Effect on the Proliferation of the Responder Cells Alone

Treg cells and PBMCs were co-cultured in the presence of a solvent, an immunomodulatory compound or a negative control article and activated for 4 days with ImmunoCult™ Human CD3/CD28 T Cell Activator in ImmunoCult™-XF T Cell Expansion Medium. The (A) suppression response at the 1:2 Treg:PBMC ratio was 45% for the solvent control, 37% for the negative control article and 89% for the immunomodulator. Co-culturing with the conventional $CD4^+CD25^-$ T cells showed < 10% of the suppression response (mean ± SD, n = 3, single donor). (B) Shown are control experiments demonstrating the proliferation of responder cells in the absence of Treg cells. Cells were cultured in conditions as described above. The immunomodulator alone had no effect on responder cell proliferation (mean ± SD, n = 3, single donor).

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Our Assay Services Workflow

1. Confidential, one-on-one consultation with our expert scientific staff.
2. Preparation of a proposal for custom-designed studies to meet your specific requirements, including a clearly defined project scope, timeline and cost.
3. Experimental execution and data analysis with STEMCELL Technologies' industry-standard reagents.
4. Preparation of a complete final report including a study summary, concise details of experimental design, tabulated data and figures, statistical analyses and photographic records.

ImmunoCult™ for T Cell Research

Contract Assay Services performs custom assays with ImmunoCult™ products designed for:

- Robust T cell activation and expansion without the use of magnetic beads, feeder cells or antigens.
- Consistent T cell expansion in serum- and xeno-free or animal component-free medium.
- Differentiation of Th1, Th2 or Treg cells.

Recommended Products

PRODUCT	DESCRIPTION	CATALOG #
EasySep™ Human $CD4^+CD127^{low}CD25^+$ Regulatory T Cell Isolation Kit	• Obtain highly viable, functional cells without the need for columns	18063
ImmunoCult™ Human CD3/CD28 T Cell Activator	• Soluble reagent for robust activation and expansion without magnetic beads, feeder cells or antigens.	10971
ImmunoCult™-XF T Cell Expansion Medium	• Rapidly expand T cells without the use of serum • Consistent expansion of functional human T cells	10981

If you are interested in learning more about Contract Assay Services at STEMCELL Technologies, visit **www.contractassay.com** or contact us at **contractassay@stemcell.com**.

References

1. Allan et al. (2008) Immunol Reviews 223(1): 391-421.