HUMAN REGULATORY T CELL ISOLATION IN 55 MINUTES USING EASYSEP™ RELEASABLE RAPIDSPHERES™

Andy I. Kokaji[1], C. Ann Sun[1], G. Neil MacDonald[1], Samuel J. Clarke[1], Steven M. Woodside[1], Maureen A. Fairhurst[1], Allen C. Eaves[1,2] and Terry E. Thomas[1]

[1] STEMCELL Technologies Inc. Vancouver, BC, Canada. [2] Terry Fox Laboratory, BC Cancer Agency. Vancouver, BC, Canada.

The preferred marker for identifying regulatory T cells (Tregs) is FOXP3 but its intracellular localization currently precludes it from being used for isolating viable cells. Human $FOXP3^+$ Tregs are characterized as $CD4^+$ T cells expressing high levels of CD25 and low levels of CD127. We have developed a novel immunomagnetic approach for the isolation of magnetic-particle-free human Tregs from PBMCs in as little as 55 minutes. This offers a vast improvement in speed over other immunomagnetic Treg isolation approaches, which typically require more than 2 hours. The standard approach for isolating Tregs combines $CD4^+$ T cell negative enrichment followed by CD25 positive selection. By contrast, our novel approach is to first perform the CD25 positive selection step with EasySep™ Releasable RapidSpheres™, a new type of magnetic particle that can be rapidly released from the isolated cells with a no-incubation, no-wash protocol performed at room temperature. Subsequent depletion of $CD127^{high}$ and non-$CD4^+$ T cells is achieved by negative enrichment with EasySep™ Dextran RapidSpheres™. Starting with $5x10^7$ PBMCs, a purity of 87.6% ± 3.1% and recovery of $2.7x10^5$ ± $8.2x10^4$ Tregs ($CD4^+CD127^{low}CD25^+FOXP3^+$) can be achieved (*n*=6). EasySep™ isolated Tregs can be expanded *ex vivo* using our novel ImmunoCult™ CD3/CD28/CD2 T cell Activation and Expansion Supplement and ImmunoCult™-XF, a serum- and xeno-free culture medium. Taken together, this is the first EasySep™ kit to incorporate the EasySep™ Releasable RapidSpheres™ and it exemplifies the potential of this technology for use in rapidly isolating functional, magnetic particle-free cell populations possessing complex cell surface phenotypes.