Rev. 2

Rat Splenocytes (RS)

Catalog #R5540

Cell Specification

The spleen stores and purifies erythrocytes, metabolizes hemoglobin, and recycles iron. The spleen also provides a critical function for the immune system by mounting a primary immune response to antigens in the blood and synthesizing antibodies [1]. Splenocytes are mononuclear cells derived from the spleen and include T-lymphocytes, B-lymphocytes, NK-cells, and NK T-cells. Primary rat splenocytes (RS) can be used to isolate CD4+ T-cells, CD8+ T-cells, and CD45R+ B cells.

RS from ScienCell Research Laboratories are isolated from normal adult rat spleen. RS are depleted of splenic macrophages, cryopreserved directly after isolation, and delivered frozen. Each vial contains at least 10 million cells in 1 ml volume. RS are quality control tested for viability. RS are negative for mycoplasma, bacteria, yeast, and fungi. RS can be maintained for a limited period of time in culture using the conditions provided by ScienCell Research Laboratories and *are not intended for long-term culture*.

Recommended Medium

It is recommended to use HematoGro Medium (HemGM, Cat. #5501) for short-term maintenance of RS *in vitro*.

Product Use

RS are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

References

[1] Mebius R, Kraal G. (2005) “Structure and function of the spleen.” *Nat Rev Immunol* 5(8): 606-616.

Instructions for culturing primary cells

Caution: Cryopreserved primary cells are very delicate. Thaw the vial in a 37ºC water bath and return the cells to culture as quickly as possible with minimal handling! Do not centrifuge the cells after thawing as this can damage the cells.

Note: *Experiments should be well organized before thawing RS. It is recommended that RS are purified or used for experiments as quickly as possible after thawing the cells. Cells are not intended for long term culture.*

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37°C, 5% CO_2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Prepare complete medium (HemGM, Cat. #5501). Thaw HemGS and P/S solution at 37°C. Gently tilt the tubes several times to ensure the contents are completely mixed before adding to the medium. Spray the medium bottle and tubes with 70% ethanol, and wipe to remove excess liquid. In a sterile field, remove the caps without touching the interior threads with fingers. Add HemGS and P/S solution to the medium and mix well.
2. Add 20 ml of complete medium to a T-75 flask. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
3. Place the frozen vial in a 37°C water bath. Hold and rotate the vial gently until the contents completely thaw. Remove the vial from the water bath promptly, wipe it down with 70% ethanol and transfer it to the sterile field.
4. Remove the cap carefully without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, culture vessel.

   Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture.
5. Replace the cap or lid, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to allow gas exchange.
6. Return the culture vessel to the incubator.
7. Cells should be promptly purified and used to isolate CD4+ T-cells, CD8+ T-cells and CD45R+ B cells.

Note: We do not recommend cryopreservation of primary cells by the end user. Refreezing cells may damage them and affect cell performance. ScienCell does not guarantee primary cells cryopreserved by the end user.

Caution: Handling animal-derived products is potentially biohazardous. Always wear gloves and safety glasses when working with these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1] Grizzle WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods*. 11: 191-9.