



## **Isolation of Cells from Spleen and Lymph Nodes**

### 1.0 Purpose

The purpose of this procedure is to document the process by which the Organ Procurement and Pathology Core (nPOD-OPPC) isolates and freezes cells from the spleen and lymph nodes.

### 2.0 Application/Scope

This procedure shall be applied to all fresh tissue processed in the nPOD Organ Procurement and Processing Core for cell extraction, excluding autopsy cases.

### 3.0 Definitions

#### 3.1 Location

The location of the nPOD OPCC is:

Organ Procurement and Pathology Core (OPPC)  
Room M621  
Department of Pathology, Immunology, and Laboratory Medicine  
University of Florida  
1600 SW Archer Road  
Gainesville, FL, USA 32610

### 4.0 Associated SOPs

- 5.1 nPOD1 Shipping and Handling Procedures
- 5.2 nPOD3 Organ Processing and Histopathology

### 5.0 Personnel and Responsibilities

#### 5.1 Martha Campbell-Thompson, PhD

Director, JDRF nPOD Organ Processing and Pathology Core  
University of Florida College of Medicine  
Department of Pathology, Immunology and Lab Medicine  
1600 S.W. Archer Rd, Box 100275  
Gainesville, FL 32610

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Fax: 352-273-7753

## 5.1.1 Responsibilities

5.1.1.1 Determine if cell isolation is going to be performed.

5.2 Maria Fernanda Martino, DMD  
Lab Manager, OPPC  
Room M621, P.O. Box 100275  
University of Florida College of Medicine  
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### 5.2.1 Responsibilities:

5.2.1.1 Perform cell isolation in a timely manner according to SOP.

5.2.1.2 Store and record information in the database.

5.2 Additional contact information is noted on the nPOD website: [www.jdrfnpod.org](http://www.jdrfnpod.org).

## 6.0 Materials and Equipment

6.1 Sterile 1x Dulbecco's Phosphate Buffered Saline (DPBS; Mg<sup>++</sup>Ca<sup>++</sup>-free. Invitrogen/Gibco, 500mL, Cat No 10010-023 or , 1 L, Cat No. 14190-144)

6.2 Cell Culture Freezing medium-DMSO; (GIBCO BRL, Cat No. 11101-01)

6.3 Cryo 1°C freezing container ( "Mr. Frosty"; NALGENE Cat No. 5100-0001)

6.4 Isopropyl Alcohol, 70%

6.5 Trypan Blue (StemSep)

6.6 CryoVials (Fisher)

6.7 Centrifuge tubes 50 mL conical. (Fisher or Sigma)

6.8 Sterile Graduated pipettes (i.e., 2,5,10,25,50mL)

6.9 Centrifuge (swinging bucket rotor) capable of maintaining 18-26°C temperature and generating 250-800 x g-force. Centrifuge must have a brake 'on/off' switch.

6.10 Hemocytometer and coverslips

6.11 Light Microscope

6.12 DPBS

## 7.0 Procedure

7.1 Keep tissue (Spleen, PLN or nonPLN) at 4C for 24 hours before starting cell isolation.

7.2 Infiltrate the tissue with cold RPMI completed media using a 1cc syringe.

<b>JDRF nPOD Standard Operating Procedures</b>	
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Original Effective Date: 8/1/2007	
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- 7.3 Proceed to mash the tissue with the tip of the syringe against a 60um sterile mesh, washing with RPMI constantly.
- 7.4 Complete volume up to 30ml and centrifuge at 500 rpm during 10 minutes at 4C.
- 7.5 Discard media and dissolve pellet in 30ml of RPMI. Repeat Centrifuge procedure.
- 7.6 Proceed to lyse Red Blood Cells as necessary using 1x solution of:
- 7.7 Incubate at 4C during 5 minutes.
- 7.8 Complete with media up to 30ml and centrifuge again
- 7.9 Discard the media, resuspend the pellet in 30ml RPMI and centrifuge with the same settings described in step 5.
- 7.10 Discard RPMI, resuspend pellet in 1xPBS, 3ml and proceed to count number of viable cells.
- 7.11 Centrifuge the cells down at 225 x g for 10 minutes to remove DPBS. Discard the supernatant (i.e., DPBS).
- 7.12 Loosen the cell pellet by gently tapping the tube. Suspend the cells in freezing medium to achieve a  $1 \times 10^7$  cells/mL cell concentration. Add the freezing medium slowly (e.g., drop wise) with constant shaking/stirring motion of the tube for proper mixing of the cells. Note: Freezing medium should be at room temperature before adding.
- 7.13 Distribute the cell suspension into appropriately labeled cryovials in 1mL aliquots.
- 7.14 Place vials in the Cryo 1°C freezing container ("Mr. Frosty") and store in a -70°C freezer overnight.
- 7.15 Next day (morning), transfer sample vials from the "Mr. Frosty" container into Liquid Nitrogen for long-term storage.

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