ONE LAMBDA, INC.
21001 Kittridge Street, Canoga Park, CA 91303-2801 USA Tel: +1 (818) 702-0042 Fax: +1 (818) 702-6904 www.onelambda.com

P R O D U C T I N S E R T

LYMPHO-KWIK® LYMPHOCYTE ISOLATION REAGENTS B, T, T/B, AND MONONUCLEAR (MN)

REF

Reagent Catalog #s: LK-50B, LK-50T, LK-50TB, LK-50MN, LKTB25, LKT25

For General Laboratory Use.

INTENDED USE

Lympho-Kwik® provides a simple procedure for the isolation of lymphocytes for use in the HLA typing assay.

SUMMARY AND EXPLANATION

Lympho-Kwik® is a premixed cocktail of monoclonal antibodies, complement and a stable density gradient developed for isolation of specific lymphocyte populations. This method of cell isolation assures maximum cell yield and purity while drastically reducing technician time. The procedure consists of a short incubation followed by a centrifugation.

PRINCIPLE(S)

Unwanted cells are lysed by monoclonal antibodies and complement. The lysed cells are then separated from the lymphocytes by density centrifugation.

REAGENTS

A. Identification

All Lympho-Kwik® reagents contain complement, a density gradient, and anti-RBC, anti-granulocyte monoclonal antibodies.

- Lympho-Kwik® B also contains anti-monocyte and anti-T-lymphocyte monoclonal antibodies.
- Lympho-Kwik® T also contains anti-monocyte and anti-B-lymphocyte monoclonal antibodies.
- Lympho-Kwik® T/B also contains anti-monocyte monoclonal antibodies.

The monoclonal antibodies are all of mouse origin.

B. Warning or Caution

1. All blood products should be treated as potentially infectious. Source material from which this product was derived was found negative when tested in accordance with current FDA required tests. No known test methods can offer assurance that products derived from human blood will not transmit infectious agents.
2. Refer to the Material Safety Data Sheet for detailed information.

C. Instructions for Use

See "Directions for Use."

D. Storage Instructions

Store reagents at temperature indicated on package. Use before printed expiration date.

E. Purification or Treatment Required for Use

1. For all Lympho-Kwik® Reagents:
   a. ***Do not refreeze Lympho-Kwik® more than once!***
   b. Thaw Lympho-Kwik® in cold tap water. Use immediately.
   c. Keep reagent immersed in ice after thawing to insure maximum reagent activity.
   d. Lympho-Kwik® may be filtered through a 0.2 micron filter.
   e. Refer to “Directions for Use” on page 2 or page 3, using whichever is applicable.
2. For all Lympho-Kwik® isolations:

   Follow instructions above and aliquot into 0.8 ml samples. Use immediately or store at -65º C or below.

F. Instability Indications

Do not use if precipitate is observed.

INSTRUMENT REQUIREMENTS

None.

SPECIMEN COLLECTION AND PREPARATION

A. **Specimen Collection Procedure**

Approximately 10 ml of whole blood should be drawn. The preferred anticoagulant is ACD or CPDA. Sodium Heparin can be used.

Caution: *Do not use lithium heparin.*

B cells should be isolated within 24 hours to achieve the highest yield. However, blood up to 3 days old can be used. Store blood samples horizontally at room temperature until beginning the isolation procedure.

TROUBLESHOOTING SPECIMEN PREPARATION

Problems	Solutions
Excessive buffy coat.	If greater than 0.1 ml buffy coat has been drawn, centrifuge lymphocytes in PBS at 1500 g for 1.5 minutes. Discard supernatant, and transfer only the white layer to a Fisher tube containing 0.8 ml of Lympho-Kwik®. Then, continue normal procedures.
Excessive red cells. Cloudy supernatant indicates excessive red cell contamination.	This may be due to inadequate incubation time or low incubation temperature. Check both time and temperature, and repeat the procedure with another 10 minute incubation.
Red cell contamination after Ficoll separation.	If excessive after Ficoll separation, OLI recommends eliminating the red cells by either lysing with ammonium chloride solution or agglutination with appropriate anti-red cell antibody. Smaller amounts of red cells should lyse with Lympho-Kwik® reagents.
Excessive granulocytes.	This may indicate overloading the reagent with buffy coat. Re-treat pellet with Lympho-Kwik®, start at step 1c of cell isolation procedures.
Excessive platelets.	Centrifuge the lymphocytes for 1 minute at 750 g. Remove supernatant, resuspend in McCoy's.
Clumped red cells or granulocytes during wash.	Centrifuge the lymphocytes for 1 second at 1000 g. Transfer the supernatant (lymphocytes) to another tube.
For Lympho-Kwik® B Reagents Only:	
Excessive background. B cell yield is greater than 20% of whole lymphocyte yield.	• Samples should be no older than two days. • The initial whole lymphocyte preparation should be clean. Excessive contamination by red cells and granulocytes weakens B cell isolation reagent activity. • Incubate at 37° C, higher temperatures cause damage to B cells. • Use no more than 10 x 10^6 whole lymphocytes. More cells overload the reagent. Corrective procedure: repeat dosage of **Reagent 1** and **Reagent 2**.

PROCEDURE

A. Materials Provided

Lympho-Kwik®

B. Materials Required, But Not Provided

1. Pipettes
2. Heat block or water bath
3. Fisher tubes or 5 ml tubes
4. Fisher microcentrifuge and/or Beckman TJ6
5. Phosphate Buffered Saline (PBS)
6. McCoy's Medium with 0.5% heat inactivated fetal calf serum (HIFCS)

C. Step-by-step procedure.

See "Directions For Use" on the next page.

DIRECTIONS FOR USE

FOR LYMPHO-KWIK® T, T/B, MN

Note: The procedure for isolation of T, T/B and MN lymphocytes using Lympho-Kwik® are identical <u>except</u> for the incubation times noted below.

A. Reagent Reconstitution
For Reconstitution Instructions, see "Reagents" section above.

B. Cell Isolation Procedures

1. Fisher Tube Method (for final yield up to 3 x 10^6 lymphocytes):
   a. Centrifuge 5 - 15 ml of whole blood (citrated or heparinized) at 400 - 900 g for 10 minutes.
   b. Resuspend 0.1 ml of buffy coat in 1 ml of phosphate buffered saline (1X PBS) and centrifuge for 1 minute at 1000 g. Discard supernatant completely.

   <u>Note</u>: It is important to start with <u>no more</u> than 0.1 ml of buffy coat, otherwise "overloading" may occur. *Overloading results in contamination of lymphocyte preparation.*

   c. Add 0.8 ml of Lympho-Kwik®, mix well, and incubate 15 minutes for MN, 30 minutes for T/B, and 20 minutes for T at 37°C in a water bath or heat block. Occasionally mix by inverting capped tube.
   d. Mix well with a pipette to break up clumps, layer 0.2 ml of PBS over cell preparation and centrifuge for 2 minutes at 2000 g. Remove and discard interface and supernatant.
   e. Resuspend pellet in PBS. Centrifuge for 1 minute at 1000 g. Discard supernatant. Repeat two times. Resuspend in McCoy's.
2. Fisher Tube Method (for final yield greater than 3 x 10^6 lymphocytes):
   a. Centrifuge 10-20 ml of whole blood (citrated or heparinized) for 10 minutes at 400 - 900 g.
   b. Transfer the entire buffy coat to 1 ml Fisher tube, and centrifuge for 1.5 minutes at 1500 g.
   c. Remove buffy coat, and distribute it equally among three Fisher tubes containing PBS.
   d. Centrifuge for 1 minute at 1000 g, discard supernatant, and re-suspend each pellet in 0.8 ml of Lympho-Kwik®.
   e. Incubate cells at 37°C in water bath or heat block for the respective time period (see step 1c above).
   f. Follow Steps 1d - 1e above.
3. Large Tube Method - 5 ml (for final yield greater than 3 x 10^6 lymphocytes):
   a. Centrifuge 10 - 20 ml of whole blood (citrated or heparinized) at 400 – 900 g for 10 minutes.
   b. Remove buffy coat and mix with equal volume of phosphate buffered saline (PBS).
   c. Layer the mixture (maximum 2 ml) over 1.5 ml Ficoll Hypaque® (D = 1.077) in 5 ml tube, and centrifuge for 10 minutes at 1000 g. Transfer the interface cells into a 5 ml tube. Centrifuge for 10 minutes at 1000 g. Discard supernatant.
   d. Resuspend in PBS. Centrifuge for 5 minutes at 1000 g. Discard supernatant. Repeat.
   e. Add 0.8 ml of Lympho-Kwik® to peripheral blood mononuclear cell (PBMC) (10 - 25 x 10^6). Mix well with pipette, and incubate at 37°C in a heat block or water bath for the respective time period (see step 1c above). Mix gently every 10 minutes.
   f. Follow Steps 1d - 1e above.
4. For use with Immunofluorescence (using any method above):

   Wash cells with 1% EDTA/PBS (pH 7.2) before final wash. This eliminates residual proteins coating the lymphocytes which may interfere with immunofluorescence testing.

   Note: *Prepare 1% EDTA with 1X PBS. Bring the pH up to 7.2 with 10 M NaOH.*

*** FOR LYMPHO-KWIK® B ***

A. **CAUTION: Please read advisories below before using Lympho-Kwik B reagents.**

- For best results, use of this B cell isolation reagent should be restricted to these sample types: Ficoll-Hypaque® isolated lymphocytes, fresh samples (less than 2 days old), Diabetic samples, Hodgkin's disease samples, non-leukemic cancer samples, cadaveric donor samples, and kidney patient samples.
- One Lambda recommends that this B cell isolation reagent NOT BE USED for the following samples: Leukemia patient samples, Eosinophilis patient samples, lymph node samples, placental blood samples, frozen cells, old samples (more than 2 days old), and lymphocytes isolated with Lympho-Kwik T/B or MN Cell Isolation Reagents.

- Only use Reagents #1 and #2 from the same lot number. See "Purification or Treatment Required for Use" above for reagent instructions.
- B cells represent 10-15% of the whole lymphocyte population in healthy adults. If the B cell yield is greater than 20% of the whole lymphocyte yield after treatment with Lympho-Kwik® B Cell Isolation Reagent, T cell contamination can be suspected.

B. Reagent Reconstitution

For Reconstitution Instructions, see "Reagents" section above.

C. Cell Isolation Procedures

1. For the isolation of 0.5 - 2 x 10^6 B Lymphocytes:
   a. Isolate no more than 10 x 10^6 whole lymphocytes by method of choice, preferably Ficoll-Hypaque®.
   b. Centrifuge the lymphocytes in a Fisher tube at 1000 g for 1 minute. Discard supernatant completely.
   c. Add 0.8 ml of **Reagent 1** and mix well.
   d. Incubate at 37°C for 60 minutes in a heat block or water bath; mix occasionally by inverting capped tube.
   e. Layer 0.2 ml of normal PBS or similar medium on top of **Reagent 1**.
   f. Centrifuge at 2000 g for 2 minutes. Remove interface and supernatant.
   g. Add 0.5 ml of **Reagent 2**. Mix well.
   h. Centrifuge at 2000 g for 2 minutes.
   i. Discard supernatant, and re-suspend lymphocytes with normal PBS; then centrifuge at 1000 g for 1 minute. Discard supernatant. Repeat two times.
   j. Resuspend in McCoy's medium.

2. Cadaveric Donor and Kidney Patient Protocol:

   Note: *With this method,* ***Reagent 2*** *is not needed.*
   a. Centrifuge 5 – 10 million whole lymphocytes @ 1000g for 5 minutes.
   b. Add 0.8 ml of **Reagent 1**, and mix well.
   c. Incubate at 37°C for 30 minutes.
   d. Centrifuge at 2000 g for 2 minutes. Discard supernatant.
   e. Add another 0.8 ml of **Reagent 1**, and mix well.
   f. Incubate at 37°C for 30 minutes.
   g. Layer 0.2 ml of normal PBS or similar media on top of **Reagent 1**.
   h. Centrifuge at 2000 g for 2 minutes.
   i. Discard supernatant, and re-suspend lymphocytes with PBS, then centrifuge for 1 minute at 1000 g.
   j. Resuspend lymphocytes in McCoy's medium. Adjust to working concentration.

3. For use in flow cytometric crossmatching.

   Note: *Use of cells isolated with Lympho-Kwik® may result in a false negative flow cytometric crossmatch. Perform the following steps to prevent false negatives.*
   a. Incubate cells isolated with Lympho-Kwik® in RPMI containing 10% HIFCS for 30 minutes at 37º C.
   b. Centrifuge cells for one minute at 1000 g. Discard supernatant.
   c. Wash 2X with PBS.
   d. Resuspend lymphocytes in RPMI containing 1% HIFCS and 0.1% sodium azide (NaN_3).

RESULTS

Lymphocytes are obtained by negative selection in which unwanted cells are lysed. The result is an enriched population of the desired lymphocytes.

LIMITATIONS OF THE PROCEDURE

The cell yield may vary with each specimen depending on the cell count and the time elapsed since blood collection. Various diseases can cause a decrease in the lymphocyte yield. Some medications can cause a decrease in the lymphocyte yield as well, and may cause a decrease in HLA antigen expression. Contamination with other cells can cause weak or false negative reactions . Monocytes have a variable amount of HLA Class I and Class II antigens. Platelets have HLA class I antigens which can weaken antisera by absorbing the antibodies from the antisera.

EXPECTED VALUES

Lympho-Kwik® reagents contain enough monoclonal antibodies and complement to lyse the contaminating cells in a 100 µl cell pellet.

SPECIFIC PERFORMANCE CHARACTERISTICS

The purity of the lymphocyte sample after use of Lympho-Kwik® reagents should be 90% or greater.

WARRANTY

This product is for use in the laboratory isolation of human lymphocytes for research purposes only. One Lambda is not liable for any direct or incidental damages caused by the use of lymphocytes isolated with this product in any subsequent application, mixture, medium, etc.

BIBLIOGRAPHY

Adams P, Clouse K, Orosz C. Functional capacities of T cells rapidly isolated with T-Lympho-Kwik®. Department of Surgery, Ohio State School of Medicine, Columbus, Ohio.

Bray RA, vanderMeer J, Sinclair DA, Holcomb JE. False negative flow cytometric crossmatches following the use of Lympho-Kwik®. In 22nd Annual ASHI Meeting Abstracts, 1996. Human Immunology, 49: Supplement 1.

TRADEMARKS USED IN THIS DOCUMENT

EC REP EUROPEAN AUTHORIZED REPRESENTATIVE

MDSS GMbH, Schiffgraben 41, D-30175, Hannover, Germany

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REVISION HISTORY

Revision	Date	Revision Description
9	2005/06	Add Catalog ID's LKTB25, LKT25. Remove TH lymphocytes from 'Directions for Use' section.
9A	2006/02	Delete Catalog ID LK-25T.
10	2007/04	Remove statement from "Limitations of the Procedure" section. Update MDSS address.