



Liqui-PREP™

The Next Generation of Liquid Cytology

Manual Technical Tips

TIP Number: 015

Subject: Large Volume Specimen Processing

TECHNICAL TIP OVERVIEW:

Liqui-PREP™ TIPS are intended to be guidelines for processing cytology specimens. The Laboratory Professional may use these guidelines or make modifications as needed to process a given specimen. In this section, each of the Liqui-PREP™ suggested basic steps will be addressed in detail along with some supporting publications.

These specimens come to the laboratory in large containers (containing more than 200ml of liquid) which require concentration or other specimen preparation. Examples of these type of specimens are Pleural, Abdominal, Synovial, Cardio, etc. These specimens are large in volume and most will require cleaning and some removal of red blood cells during the specimen preparation process.

SUGGESTED LGM INTERNATIONAL PRODUCTS FOR PROCESSING:

30-050B (50 processes) Liqui-PREP™ Cytology Processing Kit

- 1 Bottle Liqui-PREP™ Preservative Solution (Fill: 990ml per bottle)
- 1 Bottles Liqui-PREP™ Cellular Base Solution (Fill: 26ml per bottle)
- 1 Box of 15ml Plastic Centrifuge Tubes (100 tubes)

30-100B (100 processes) Liqui-PREP™ Cytology Processing Kit

- 2 Bottle Liqui-PREP™ Preservative Solution (Fill: 990ml per bottle)
- 2 Bottles Liqui-PREP™ Cellular Base Solution (Fill: 26ml per bottle)
- 1 Box of 15ml Plastic Centrifuge Tubes (100 tubes)

30-100B1 (100 processes) Liqui-PREP™ Cytology Processing Kit

- 2 Bottle Liqui-PREP™ Preservative Solution (Fill: 990ml per bottle)
- 2 Bottles Liqui-PREP™ Cellular Base Solution (Fill: 26ml per bottle)

40-100B (100 processes) Liqui-PREP™ Special Processing Kit

- 1 Bottle Liqui-PREP™ Preservative Solution (Fill: 990ml per bottle)
- 1 Bottle Liqui-PREP™ Cleaning Solution (Fill: 410ml per bottle)
- 2 Bottles Liqui-PREP™ Cellular Base Solution (Fill: 38ml per bottle)
- 1 Box of 15ml Plastic Centrifuge Tubes (100 tubes)

40-100B1 (100 processes) Liqui-PREP™ Special Processing Kit

- 1 Bottle Liqui-PREP™ Preservative Solution (Fill: 990ml per bottle)
- 1 Bottle Liqui-PREP™ Cleaning Solution (Fill: 410ml per bottle)
- 2 Bottles Liqui-PREP™ Cellular Base Solution (Fill: 38ml per bottle)

Any questions, contact your local Liqui-PREP™ representative or:

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SUGGESTED LGM INTERNATIONAL PRODUCTS FOR PROCESSING:

Liqui-PREP™ Useful Accessories

- 60-1000: Liqui-PREP™ Preservative Solution - 4 bottles (990ml per bottle)
- 60-2000: Liqui-PREP™ Preservative Solution - 4 bottles (1,880ml per bottle)
- 60-4000: Liqui-PREP™ Preservative Solution - 4 bottles (3,750ml per bottle)
- 70-500: Liqui-PREP™ Cleaning Solution - 6 bottles (410ml per bottle)
- 80-1000: Liqui-PREP™ Lytic Reagent - 4 bottles (990ml per bottle)

Specimen Collection:

These specimens are collected under clinic settings by physicians. Collection containers are varied in type and size. These specimens must be sent to the laboratory for processing immediately after collection because the specimen, due to its size, will not be preserved at collection site.

Specimen Preparation:

Note: Removal of mucous and red blood cells can be performed in the 50ml centrifuge tube during specimen preparation, in the 15ml centrifuge tube after specimen preparation or during both the 50ml and 15ml centrifuge tubes.

These large volume specimens need to be prepared by making representative aliquots. The number of aliquots to be made are determined by the laboratory. Immediately upon arrival, the specimen aliquots should be prepared.

The residual of the specimen should be held at refrigerated temperatures. The following process can be used to process most of the large volume specimens. There are two specimen preparation procedures. One for specimens with little or no red blood cells and the second for specimens with red blood cell that should be reduced to get good slides to read.

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Specimen Preparation:

Specimens having minimal to no red blood cells

- The specimen is mixed well. A combination of manual rolling of the specimen or a rotating mixer for 10 to 30 minutes is used. It is not unusual to prepare more than 1 aliquot of specimen for processing of these specimens.
- Pipette approximately 8ml of Liqui-PREP™ Cleaning Solution into a 50ml centrifuge tube.
- 20ml of Liqui-PREP™ Preservative Solution is pipetted into a 50ml centrifuge tube.
- 20ml of the well mixed specimen is poured into the 50ml centrifuge tube.
- The 50ml centrifuge tube is mixed well and allowed to preserve for at least 1 hour.
- Centrifuge the 50ml centrifuge tube at 1000xg for approximately 10 minutes.
- Decant the supernatant. Then mix the pellet well.

NOTE: These pellet can be rather large. It is suggested, but not required, to split these large pellets into additional aliquots of 200µl to 300µl cellular aliquots. This makes the cellular pellets easier to process. Pipette these aliquots into 15ml centrifuge tubes.

- Pipette 4ml of Liqui-PREP™ Cleaning Solution into a 15ml centrifuge tube. If splitting the cellular pellet, additional 15ml centrifuge tubes may be used.
- Pipette 8ml of Liqui-PREP™ Preservative Solution into the 15ml centrifuge tubes and mix well by inverting.

Specimen Cleaning and/or Concentration:

After specimen preparation, all of these specimens are in 15ml centrifuge tubes. The specimen centrifuge tubes are centrifuged at 800xg for approximately 10 minutes.

After centrifugation, the supernatant is decanted by inverting the centrifuge tube.

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Cellular Encapsulation and Slide Production:

“Working Tube” - The use of a “Working Tube” is the simplest and most consistent method of diluting and encapsulating the cells from a cell pellet. Any small test tube can be used as the “Working Tube”. This is also the most efficient use of Liqui-PREP™ Cellular Base Solution.

- Pipette 300µl to 500µl of Liqui-PREP™ Cellular Base Solution into a working tube. The amount of Liqui-PREP™ Cellular Base Solution to be used depends on the cellularity of the slide favored by the end user. LGM International, Inc. uses 400µl of Liqui-PREP™ Cellular Base Solution.
- After mixing the cellular pellet well to produce a homogeneous suspension, pipette 50µl of the cellular suspension into the “Working Tube”.
- Mix the “Working Tube” well using a vortex mixer.
- Withdraw a 50µl aliquot from the well mixed “Working Tube”. Dispense and apply this 50µl aliquot onto a Microscope slide.
- Allow the slides to dry, stain and read.

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Specimen Preparation:

Specimens Needing red blood cell removal

- The specimen is mixed well. A combination of manual rolling of the specimen or a rotating mixer for 10 to 30 minutes is used. It is not unusual to prepare more than 1 aliquot of specimen for processing of these specimens.
- Pipette approximately 8ml of Liqui-PREP™ Cleaning Solution into a 50ml centrifuge tube.
- 10ml of Liqui-PREP™ Preservative Solution is pipetted into a 50ml centrifuge tube.
- 10ml of Liqui-PREP™ Lytic Reagent is pipetted into the 50ml centrifuge tube.
- 20ml of the well mixed specimen is poured into the 50ml centrifuge tube.
- The 50ml centrifuge tube is mixed well and the centrifuge tube is allowed to both preserve and lyse red blood cells for no more than 10 to 15 minutes.
- Centrifuge the 50ml centrifuge tube at ~1000xg for approximately 10 minutes.
- Decant the supernatant. Then mix the pellet well.
- Pipette 8ml to 10ml of Liqui-PREP™ Preservative Solution into the 50ml centrifuge tube.
- Mix the specimen well and allow to preserve for at least 1 hour prior to continuing with specimen preparation.
- Centrifuge the 50ml centrifuge tube at 1000xg for approximately 10 minutes.
- Decant the supernatant. Then mix the pellet well.

NOTE: These pellet can be rather large. It is suggested, but not required, to split these large pellets into additional aliquots of 200µl to 300µl cellular aliquots. This makes the cellular pellets easier to process. Pipette these aliquots into 15ml centrifuge tubes.

- Pipette 4ml of Liqui-PREP™ Cleaning Solution into a 15ml centrifuge tube. If splitting the cellular pellet, additional 15ml centrifuge tubes may be used.
- Pipette 8ml of Liqui-PREP™ Preservative Solution into the 15ml centrifuge tubes and mix well by inverting.

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- Mix the “Working Tube” well using a vortex mixer.
- Withdraw a 50µl aliquot from the well mixed “Working Tube”. Dispense and apply this 50µl aliquot onto a Microscope slide.
- Allow the slides to dry, stain and read.

References:

- “Immunophenotyping of malignancy and MCMII expression in pleural effusion”, M. Southwood; J. Buckwell; C. Atkinson, Department of Pathology, Papworth Hospital NHS Foundation Trust, Cambridge, United Kingdom
- “Liquid-Based Cytology - next generation technology”, M. Southwood; A. Brighty; J. Geyer, Department of Pathology, Papworth Hospital NHS Foundation Trust, Cambridge, United Kingdom, Vector Laboratories, United Kingdom.

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