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Thermo Scientific Shandon Cytoblock Cell Block Preparation System Instructions for Use

For in vitro diagnostic use.
For use as a kit to facilitate the preparation of paraffin-embedded cell suspensions.

Note: Please read instruction manual carefully before using this kit. The gel produced is not compatible with fixatives containing phosphates. Unbuffered formal saline or Thermo Scientific™ Shandon™ Formal-Fixx™ is recommended.

The Thermo Scientific™ Shandon™ Cytoblock™ system is designed to facilitate the preparation of paraffin-embedded cell suspensions, cell aggregates, and tissue fragments. The Cytoblock system simplifies the production of paraffin blocks from cellular material, and increases the yield of useful blocks from cellular suspensions and cell aggregates. The Cytoblock system can also be used to process tissue biopsies and fragments which are difficult or impossible to process using other techniques. Preparation of cell blocks with the Cytoblock system is so simple and reliable that retention of a paraffin-embedded block for every cytological sample is a practical laboratory procedure.

Cytoblock can be used to produce paraffin-embedded blocks from fine needle aspirates, cutting needle cores, body fluids, and residual sediment from other cytological preparations. Cytoblock is also an ideal method for processing tissue fragments such as small biopsies, curettings, and other specimens which are too small to be processed in standard cassettes. The use of the Cytoblock system eliminates the need for teabags, tissue wrapping, and the potential loss of tiny fragments.

The Cytoblock Kit

The Cytoblock kit contains:
50 Cytoblock Cassettes - complete with backing papers and board inserts
1 Cytoblock Reagent 1 (clear fluid)
1 Cytoblock Reagent 2 (colored fluid)

The Cytoblock cassettes come preassembled with backing papers and board inserts in place. The Cytoblock kit components are designed to be used with the Thermo Scientific™ Cytospin™ to process cell suspensions and aggregates. The Cytoblock kit components can be used alone to process tissue fragments and biopsies. After processing the Cytoblock, the Cytoblock cassette is used to form a paraffin base, with clamping dimensions identical to a standard tissue cassette. The Cytoblock cassette can be used with standard cassette base molds during paraffin embedding.

Using Cytoblock to Prepare Cell Blocks

Cytospin preparations of cytological specimens

1. Record patient information on Cytoblock.
2. Specimen should be fixed prior to beginning Cytoblock preparation in the Cytospin.
3. Concentrate the fixed cells by centrifugation, pour off excess fluid and drain.
4. Estimate amount of specimen present. If the total amount of specimen is 2 drops or less, add 4 drops of Reagent 2 to the specimen pellet and mix by vortexing or by repeated aspiration. If the specimen quantity is larger than 2 drops, you will have to decide if you wish to make more than one block. Each block should have 2 drops of specimen or less. If you have enough specimen for two blocks, you can add 8 drops of Reagent 2 and mix by vortexing. [The mixture will be divided equally between two (2) Thermo Scientific™ Shandon™ Cytofunnel™ disposable sample chambers at step 9.]
5. Assemble Cytoblock cassette into Thermo Scientific™ Shandon Cytoclip™, with Cytoclip horizontal. The locating peg on the back of the Cytoblock cassette fits into the hole in the Cytoclip to ensure proper orientation.
6. Apply 3 drops of Reagent 1 into the center of the well in the board insert. Reagent 1 should coat the entire circumference of the well in the board insert. Use care to avoid any Reagent 1 on the top surface of the board insert.
7. With the backing paper projecting toward the top of the Cytoclip, place a Cytofunnel disposable chamber over the prepared Cytoblock and secure the metal clip holder in the usual manner.
8. Place the assembled Cytoclip into the Cytospin Sealed Head.
9. Place the mixed cell suspension in each Cytofunnel.
10. Close the Cytospin and set for five (5) minutes at 1500 rpm. Use the LOW acceleration setting. Start Cytospin.
11. When the Cytospin stops, remove the Cytofunnel assemblies and place horizontally. Release the clip and remove the funnels. Removal is facilitated by rocking the funnel to the side to separate the funnel assembly from the underlying board insert. Be certain the cell button is in the well, and has not adhered to the funnel. Discard the funnel.
12. Place one drop of Reagent 1 in the center of the insert board well, on top of the cell button. Close the Cytoblock cassette and place in fixative to await processing.

    Note: Fixative should be UNBUFFERED (try Formal-Fixx™) or the first processing alcohol.

    Warning: DO NOT USE PHOSPHATE-BUFFERED solutions during any processing step.
13. Process cassettes in standard tissue processor.
14. At embedding, open Cytoblock cassette. Fold back paper and remove board insert. The board insert can be removed easily from the Cytoblock cassette by inserting the tips of fine forceps through any of the holes under the insert.
15. Dislodge cell button into base mold and embed flat. Discard the board insert and backing paper.
16. Re-close Cytoblock cassette and place FLAT SIDE UP (round peg side down) on top of base mold. Fill with paraffin.
17. Handle as with any paraffin block. Use caution when sectioning, since the cell button is thin and can be quickly used up by excessive trimming.

NOTE: Sections derived from Cytoblock embedded specimens retain the gel matrix around the cells. This matrix may stain faintly with some stains. It can be completely removed by a brief (3 to 5 minutes) rinse in phosphate-buffered saline (PBS, pH 6.8-7.2) prior to staining (but after removal of paraffin). A formulation for PBS is included in the appendix to these instructions.

Cytoblock preparations from biopsies and tissue fragments.

1. Specimen should be fixed prior to beginning Cytoblock preparation.
2. Place a Cytoblock cassette with backing paper and board insert horizontally on toweling.
3. Apply 3 drops of Reagent 1 into the center of the well in the board insert.
4. Arrange tissue fragments in the board well. The surface tension of Reagent 1 will permit specimens to be oriented as desired.
5. Add sufficient quantity of Reagent 2 to completely fill the well containing the tissue, no more than 4 drops. You may perform further orientation of specimens as the gel begins to solidify. This must be done quickly, before the gel becomes too rigid.
6. Put one drop of Reagent 1 in the center of the insert well, on top of the gel button. Close the Cytoblock cassette and place in fixative to await processing.

    Note: Fixative should be UNBUFFERED (try Formal-Fixx) or the first processing alcohol.

    Warning: DO NOT USE PHOSPHATE-BUFFERED solutions during any processing step.
7. Process cassettes in standard tissue processor.
8. At embedding, open Cytoblock cassette. Fold back paper cover and remove board insert. The insert may be removed by inserting the tips of fine forceps through the holes in the back of the Cytoblock cassette under the insert.
9. Dislodge gel button containing tissue fragments and place into embedding mold. Discard the board insert and the backing paper.
10. Re-close Cytoblock cassette and place FLAT SIDE UP (round peg side down) on top of the base mold. Fill with paraffin.
11. Handle as a standard paraffin block.

Note: Sections derived from Cytoblock embedded specimens retain the gel matrix around the tissue fragments. This matrix may stain faintly with some stains. It can be completely removed by a brief (3 to 5 minutes) rinse in phosphate-buffered saline (PBS, pH 6.8-7.2) prior to staining (but after removal of paraffin).

Trouble-Shooting Guide

Inadequate cell numbers
Pre-centrifugation of cellular suspensions ensures a concentrated specimen. When few cells are present, and the specimen must be extended with Reagent 2, cells may be widely separated when the resulting mixture is placed in the Cytofunnel disposable sample chamber. Inadequate cell numbers reflect an inadequate specimen, and care must be taken to conserve as many cells as possible prior to using the Cytoblock system.

Few cells in block, but many in specimen
Cell sample should be mixed with Reagent 2 before the cell sample comes in contact with the board insert. Be certain the sample is well mixed with Reagent 2 prior to beginning cytocentrifugation. This precaution, along with care in wetting the bottom and sides of the insert well prior to assembly of the Cytoclip-funnel assembly, will ensure that cells are captured in the gel button, and do not have an opportunity to lodge in the Cytofunnel filter card or board insert prior to gel formation.

Inadequate fixation
Prior fixation of specimens before using the cell block system, plus the usual fixation action of the various processor stations should prevent any problems of inadequate fixation. However, if the specimen is not fixed until some time after collection, cell degeneration may proceed to the point where the result appears to be inadequate fixation.

Loss of integrity of gel buttons containing cells or fragments
The polymeric material is very stable to most organic solvents and hot wax. It is slowly depolymerized by chelating agents, such as EDTA, and in the presence of phosphate ions, as in phosphate-buffered formalin. Gel buttons made using the Cytoblock system should not be exposed to solutions containing these depolymerizing agents.

Specifications

Appendix

Phosphate-Buffered Saline

Sodium Chloride	7.90 g	NaH2P04 H20	0.58 g
Na2HP04	1.53 g	Distilled Water	1 L

Warnings and Precautions

See Safety Data Sheets for warnings and precautions, as well as EUH code definitions.
See container label for warnings and precautions.

Order Information

Product	Qty.	REF
Cytoblock Cell Block Preparation System, complete with: 50 Cytoblock cassettes, 1 Cytoblock Reagent No. 2, 1 Cytoblock Reagent No. 1, 1 Instruction manual	1 Kit	7401150

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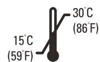

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