



Exalpa Biologicals, Inc.

NUPARAFFIN™

KIT SUMMARY FACTSHEET

NUPARAFFIN KIT CONTENTS:

CAT.# 7035

1.	1 vial Lyophilized NuParaffin Enzyme Preparation	25 Tests
2.	1 vial Enzyme Stabilizer	22.5 ml
3.	1 vial Enzyme Activator	2.5 ml
4.	25 NuCycl Sample Preparation Filters	25 Filters
5.	1 Instruction Manual	1 manual

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MATERIALS REQUIRED BUT NOT PROVIDED BY EXALPHA:

Materials needed for Exalpa's NuParaffin

- Flow Cytometry Instrument or DNA Analyzer
- Test Tubes compatible with Instrument or Analyzer
- Calibrated Liquid handling device or pipette capable of accurately delivering 1 ml of NuCycl reagent to samples and standards.
- 35 or 50 mm petri dish for tissue dissociation
- Refrigerated Centrifuge capable of 400g
- # 10 scalpel blades for mincing tissue
- Pipette for transferring specimen from petri dish to Exalpa's Special Sample Preparation Funnel Filters.
- Ice bucket

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ASSAY PROCEDURE: <<<<< NuParaffin Paraffin Extraction Kit >>>>>

The NuParaffin, Paraffin-embedded Tissue Extraction Kit allows researchers and clinicians to extract cells/nuclei from paraffin-embedded tissues with subsequent staining for DNA and/or cell surface phenotypic markers.

Assay Procedure: The following procedure is recommended for the preparation of paraffin embedded tissues for DNA Flow Cytometry or phenotypic analysis of cells from paraffin embedded sections or tissues. Set instrument or analyzer for DNA/RNA analysis. See instrument manufacturers instructions for proper DNA/RNA procedure.

Note: Exalpa recommends that the NuCycl Sample Preparation filters be used for the preparation of all samples and standards to be run. NuCycl Sample Preparation Filters remove background noise and allow for tighter CV's and hence better sensitivity than would otherwise be possible.

Sample Preparation Procedure for Paraffin Embedded Tissues:

Paraffinated Tissues On Slides

1. Fix tissues in paraformalin or formalin and embed in paraffin.
2. Cut 30 micron sections and bind to precleaned glass slides by warming. Alternately sections may be placed into clean test tubes for hydration.
3. Deparaffinate tissues by placing slides in a rack and carefully placing in xylene for 15 minutes and then hydrating through a series of ethanol's (100%, 100%, 75%, 50%, 25%, 0%, and 0%) at 10 min. intervals. This step should be done within 2 hours of enzyme treatment. If using slides, add 2 ml of xylene or each concentration of ethanol to each test tube, incubate 10 minutes and centrifuge at 400 x

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g between each step.

4. A control tissue with a known DNA index and percentage aneuploid distribution should be used. Many sections may be cut from a control tissue and be used as a control for each preparation. Most aneuploid tissues used as a control will contain a normal cellular population for use as a diploid reference standard.

Isolation of Nuclei/Cells From Deparaffinated Tissues and Staining Procedure

1. With a scalpel, carefully remove the deparaffinated tissue from the slide(s). Use two tissue sections with 5-10 mm diameter or one of equivalent size. Place tissue in one ml of NuParaffin final solution. If hydration was performed in test tubes simply add 1 ml NuParaffin solution to each test tube.

2. Incubate the tissue/NuParaffin mixture at 37°C for 30 minutes in a rocker bath (20 rpm) or mix by hand every 5 min.

3. Pass the mixture through a Pasteur pipette 5 times to aid in dissociation of tissue.

4. The nuclear suspension and debris are separated from each other using the specially designed funnel filter provided. Place a test tube in which the nuclear suspension is to be collected in a rack. Place the funnel filter into the test tube and add the sample to the top portion of the funnel. Collect the stained sample in the test tube.

NOTE: It is essential that Exalpha's specially designed funnel filters be used for all applications in order to achieve optimal results.

5. Centrifuge at 200 xg for 8 minutes in a refrigerated centrifuge set at 4°C.

6. Resuspend the pellet in 1 ml of NuCycl DAPI or NuCycl PI for DNA analysis.

7. For NuCycl DAPI and NuCycl PI, samples should be stored on ice and analyze by flow or static cytometry within one hour.

NuParaffin Reagents Provided and Preparation

NuParaffin Reagents Provided

Reagent A - A special mixture of enzymes and inorganics which are stable for shipment at refrigerated temperature (0-4°C) and should be stored at -70°C upon receipt.

Reagent B - Contains an enzyme activator - 2.5 ml - stable at room temperature

Reagent C - Contains an enzyme stabilizer - 22.5 ml - stable at room temperature

NuParaffin Reagent Preparation

1. Carefully mix Reagent A with some of reagent C (~10 ml) by adding ~ 10 ml Reagent C to the bottle containing the lyophilized Reagent A. **DO NOT** cause foaming, mix by gently rolling or inverting the vial.

2. Add slowly 2.5 ml of Reagent B to the vial containing Reagent A and C.

3. Wash out the Reagent B bottle with the remaining Reagent C and add to the vial containing Reagents A & C. The NuParaffin reagent is now ready for immediate use.

4. Use 1 ml of prepared NuParaffin per sample. If you have less than 25 samples to analyze, allow 1 ml NuParaffin reagent per sample to be analyzed. Label and date each aliquot and freeze the remaining solution as outlined under storage. NuParaffin will be stable for 3 months from the date of preparation.

PRECAUTIONS:

1. Do not use tissue sections below 30 microns in thickness. Sections of 50 microns may be used but do not, in general, adhere to slides very well.

2. Clean slides with alcohol before use. The slide surface area may be increased by placing the precleaned slides in a 1% (w/v) ammonium bifluoride solution for two minutes to increase the surface area for better adhesion. Thoroughly rinse the slides with ethanol and dry after etching.

3. Run samples in duplicate.

4. Paraffin embedded tissues should never be used in lieu of fresh, frozen samples due to

the increase in debris in paraffinated tissues which result in lower resolution.

5. Enzymes preparations are to be used immediately after thawing.

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KIT STORAGE AND EXPIRATION DATING: NuParaffin should be stored at -70°C before use, the liquid components, i.e., Reagent B and Ceagent C should be stored at room temperature until use. Once the NuParaffin reagents have been prepared, the unused portions should be aliquoted in single use, 1 ml portions, in freezer vials and stored at -70°C until needed. The unopened package of NuParaffin will be stable until the expiration date, frozen aliquots of NuParaffin will be stable for 3 months at -70°C from date of preparation.

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