FineFIX

THE NEW FORMALIN-FREE FIXATIVE
FOR OPTIMAL MORPHOLOGY
AND MOLECULAR ANALYSIS

MILESTONE
THE SEARCH FOR THE IDEAL FIXATIVE IS ON

The use of formalin as a routine fixative for histology has remained unchanged for well over a hundred years. Its wide acceptance has been mainly attributed to low cost and quality of morphology, despite its high level of toxicity.

The past two decades has seen a growing need for a non toxic formalin substitute, spurred by the need for a safer laboratory environment and the startling advances in molecular biology. Several studies have confirmed that specimens which are currently processed using aldehyde based fixation (e.g. formalin), show extensive protein cross-linking, which makes recovery of biomolecules tenuous (1).

The ideal fixative should present a low level of toxicity, produce optimal H&E, IHC and histochemical staining and allow the recovery of DNA/RNA for molecular analysis.

The two types of formalin substitutes currently available are:
1) Aldehyde based. These present a somewhat lower level of toxicity than formalin, but do not allow optimal DNA/RNA recovery,
2) Ethanol based. These fixatives have overcome the problems for recovery of DNA/RNA, but have shown less than satisfactory results for routine histology, due to shrinkage and vacuolization.

THE SEARCH IS OVER: FINEFIX

Milestone has taken advantage of its know-how from over 25 years of combined experience in the field of microwave enhanced organic chemistry and microwave applications in histology to develop a new fixative – FineFIX, formulated for conventional and microwave enhanced fixation. This highly efficient formalin-free fixative is ideal for routine histological applications and molecular analysis.

WHAT IS FINEFIX

FineFIX is a formalin-free, water-based concentrate. When diluted with ethanol, its patented formulation of low toxicity additives overcomes the drawbacks commonly associated with the use of pure ethanol or ethanol based fixatives, e.g., significant tissue shrinkage, vacuolization and pyknotic nuclei. FineFIX also provides optimal preservation of tissue antigens, nuclear and cytoplasmic morphology and reduced lysis of red blood cells with preservation of the cytoplasmic membranes.

The ethanol concentration in the working solution of FineFIX is approximately 70%. This concentration was found to produce good histology and to allow optimal recovery of DNA/RNA and proteins, sufficient for several downstream molecular analyses (1).

THE BENEFITS OF FINEFIX IN THE GROSS-ING ROOM

- Simultaneous specimen fixation, dehydration and fat extraction while immersed in FineFIX.
- Macro firming of specimens allows pathologists to easily palpate, dissect and cut thinner representative blocks.
- Easy detection of lymph nodes: an easy gray-white contrast following FineFIX fixation.
- Elimination of the slimy feel and consistency of fatty specimens for a rapid, easy clean up.
- More "real-life" color compared to "gray-ish" appearance of formalin fixed specimens.
malin without the disadvantages

**THE BENEFITS OF FINEFIX FOR HISTOPROCESSING**

- Reduction of tissue processing times by initiating earlier dehydration and fat extraction during the fixation process
- Ideally suited for rapid microwave and conventional fixation procedures
- Well suited as a post fixative, removing fat from fatty specimens such as breast
- Suitable as a formalin substitute in the first container of a conventional tissue processor

**THE BENEFITS OF FINEFIX IN THE MICROSCOPY ROOM**

How does FineFIX compare with Formalin for morphology and staining?

The morphology of FineFIX fixed tissues processed either conventionally or by microwaves is of such high quality that it poses no difficulty for diagnosis. Shrinkage and vacuolization are reduced to a fraction of what is experienced with traditional ethanol fixation. Pyknotic nuclei are also absent.

**HOSPITAL TESTED**

FineFIX has been extensively tested in a hospital laboratory environment to ensure its suitability for routine H&E, IHC and histochemical staining for diagnostic purposes, and for molecular analysis.

**H&E STAINING**

Tissues fixed in FineFIX show improved H&E staining intensity and cytological details.

- Bone marrow Formalin H-E x400
- Bone marrow FineFIX H-E x400
- Breast carcinoma FineFIX H-E x100
- Gastric epithelium FineFIX H-E x400

Examples of ethanol-based fixatives without...
**HISTOCHEMISTRY**

When using FineFIX, special stains procedures can be carried out without any change to existing protocols.

**IMMUNOISTOCHEMISTRY**

Improved Immunohistochemical staining by reducing the need for aggressive epitope retrieval.

**Prostate nodular hyperplasia FineFIX H-E x400**

**Skin melanocytic nevus FineFIX H-E x100**

**Kidney PAS stain FineFIX x100**

**Kidney reticulin stain FineFIX x200**

**Meningioma staining for vimentin x200**

**Ependymoma staining for cytokeratin CAM5.2 x200**

**Breast carcinoma staining for Mib-1 x400**

**Breast carcinoma for p-53 x200**

**HISTOCHEMISTRY**

out their disadvantages: FineFIX
MOLECULAR BIOLOGY

Although formalin-fixed and archived tissues are a huge source of DNA for molecular biological studies in cancer research, screening for genetically based diseases and developmental biology studies, attempts to extract DNA from formalin-fixed tissues for molecular biological studies have been variably successful. Formalin fixation at room temperature results in poor preservation of high-molecular weight DNA, the size of the extracted DNA being directly related to the fixation temperature. Even short-term treatment of sections with formalin have been shown to significantly reduce DNA solubility, with up to 30% of nucleic acids being lost during the fixation process.

With the expansion of PCR and other techniques for nucleic acid analysis for clinical diagnosis, an understanding of the deleterious effects of formalin as the primary method of choice, followed by recovery of preserved DNA and RNA is becoming increasingly important, as the need for molecular pathology to arrive at a conclusive diagnosis will increase in the future.

Multiple studies have indicated that the use of non-cross-linking alcoholic reagents yielded superior results as nucleic acid fixatives, rather than aldehydes.

DNA extraction from formalin-fixed, paraffin-embedded human samples and from finefix-fixed, paraffin-embedded human samples: a comparative analysis

Tissue deparaffinization and DNA extraction
Ten samples, 5 from formalin fixed and paraffin embedded human tissues (13M, 4227/01, 3479A/01, 3563/01C and 24B), and 5 from FineFIX fixed and paraffin embedded human tissues, (24L, 13F, 3B2, 49B and YR1B), were subjected to DNA extraction. Five mm-thick section were cut with a microtome from each paraffin block and placed in a 1.5 ml microcentrifuge tube. The sections were deparaffinized at room temperature. All samples were digested with Proteinase K (500 mg/ml) and SDS (1%) at 50°C for 3 hours and further subjected to DNA extraction with a commercial kit for DNA extraction.

To evaluate the amount of the purified DNA, 10 μl of the DNA solution were run on agarose gel 0.8% (Figure 1).

DNA amplification by PCR
DNA extracted from all samples were subjected to PCR amplification using a set of primer which amplify a 165 bp region of the beta globin gene.

MVI: molecular weight marker (Roche)
C+: positive control of the PCR reaction
C-: negative control of the PCR reaction

Comments:
1. DNA from FineFIX fixed tissues can be successfully extracted faster than DNA from formalin fixed specimens.
2. DNA from FineFIX tissues is not degraded and is recovered in higher amounts compared to DNA extracted from formalin fixed tissues.
3. DNA extracted from FineFIX tissues, employed for PCR analyses, gives better results in term of amplification signal for a given target sequence.

The use of FineFIX, optimizes the preservation of DNA and RNA for Molecular analysis. It is also suitable for storing specimens at minus 80°C, without hardening, allowing easy sampling.
MORE FineFIX ADVANTAGES

FineFIX is a low toxicity fixative

Airborne Exposure Limits
The allowable exposure limits of FineFIX (when reconstituted into a working solution by the addition of ethanol) versus conventional Formalin (10%).

The improvement in airborne exposure limits further stresses the advantage of a formalin free laboratory, with the ensuing benefits for staff to work in significantly safer environment, the "Green Lab"

### FineFIX Savings in Storage and Disposal
- Elimination of expensive disposal procedures such as chemical neutralization
- Recycling of the ethanol component of FineFIX can be easily carried out
- Disposal of FineFIX working solution can be carried out according to regulations for ETHANOL
- Storage requirements for FineFIX concentrate fixative are 70% less than conventional diluted fixatives

### Benefits of FineFIX in Storage and Shipping in Cold Climates
FineFIX is ideal for storage and transport of specimens in cold climates. It will not freeze at temperatures as low as minus 50°C (122°F)

### Handbook of Guidelines for the Correct Use of FineFIX
FineFIX is supplied with a handbook of guidelines to enable users to immediately operate with this fixative, avoiding any trial and error experiments. Guidelines for Epitope Retrieval procedures complete the set of information required for a smooth transition to a formalin-free laboratory.

References

ORDERING INFORMATION
FineFIX concentrate tank of 2.5 lt P/N 70147
FineFIX concentrate tank of 5 lt P/N 70148
FineFIX concentrate tank of 10 lt P/N 70149

Specifications are subject to change without notice.

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