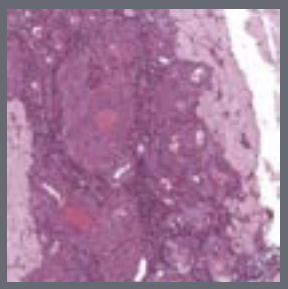
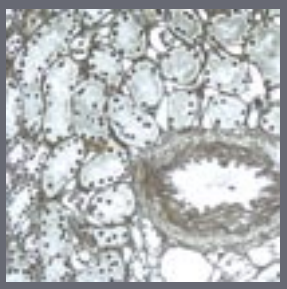
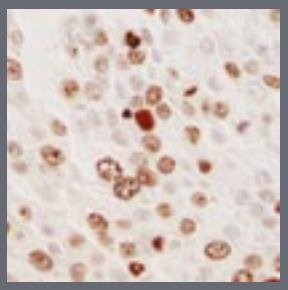
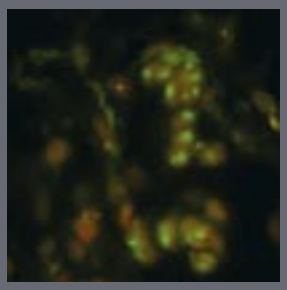
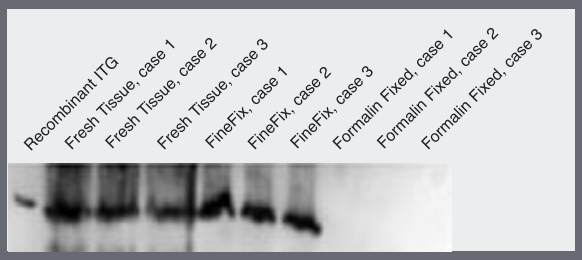
FineFIX











The New Formalin-free Fixative
for Optimal Morphology and Molecular Analysis

MILESTONE

COMPASSIONATE TECHNOLOGIES FOR SAME-DAY DIAGNOSIS

MILESTONE

A Company with a Caring Goal

Milestone is committed to reducing patients' anxiety and stress by providing pathologists and histologists with innovative technologies to enable same-day diagnostic results

THE SEARCH FOR THE IDEAL FIXATIVE IS ON...

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, Protein for molecular analysis.

Good news!
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 patented formalin-free, water-based concentrate. When diluted with ethanol, its 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.

FineFIX working solution is prepared by mixing 1 part of FineFIX concentrated to 3 parts of ethanol (98%)

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].

European Patent EP 1455174B1 (December 15th 2004)
US patent US 20050074422 (April 1st 2005)

FineFIX concentrate

2,5 liter | 5 liter | 10 liter

THE BENEFITS OF FINEFIX IN THE GROSSING 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.
- Elimination of the slimy feel and consistency of fatty specimens for a rapid, easy clean up.
- More "real-life" color compared to "grayish" appearance of formalin fixed specimens.
- Easy detection of lymph nodes: an easy gray-white contrast following FineFIX fixation.

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 [2]. 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 [3], with up to 30% of nucleic acids being lost during the fixation process [4].

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 [5].

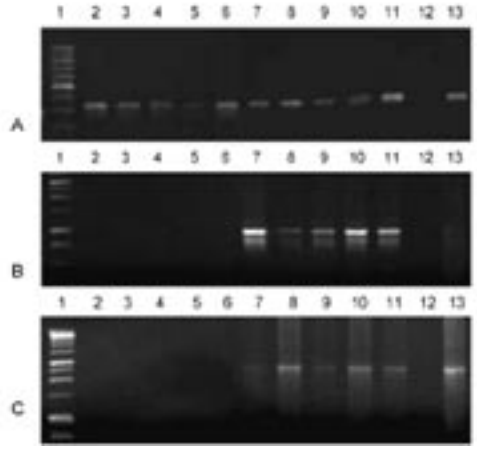
Multiple studies have indicated that the use of non cross-linking alcoholic reagents yielded superior results as nucleic acid fixatives, rather than aldehydes [6,7].

A Novel Fixative Improves Opportunities of Nucleic Acids and Proteomic Analysis in Human Archive's Tissues

Giorgio Stanta, MD, Stefano Pozzi Mucelli, MS, Francesca Petrera, MS, Serena Bonin, PhD, and Gianni Bussolati, MD

DIAGN MOL PATHOL. 2006 june 15(2): 115-23.

DNA amplification by PCR



Ten samples five from formalin-fixed and five from FineFIX-fixed tissues were subjected for DNA extraction.

250 ng of DNA of each sample has been amplified using a primer of 291 bp (data not shown), 339 bp (A), 1000 bp (B), 1939 bp (data not shown) , 2396 bp (C) region of human transthyretin gene.

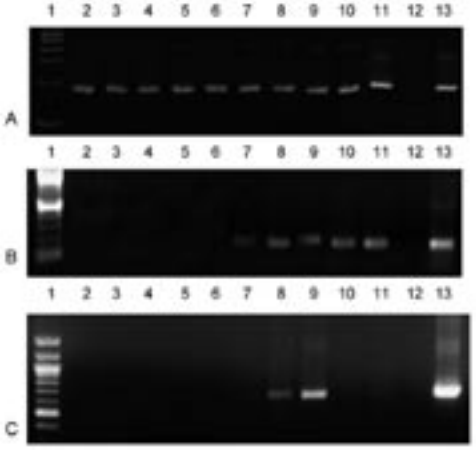
Each gel (A, B and C) shows:

Lane 1, molecular size marker; Lane 2-6, DNA obtained from formalin-fixed tissues; Lane 7-11, DNA obtained from FineFIX-fixed tissues; Lane 12, negative control; Lane 13, DNA from HepG2 cells, positive control.

DNA Amplification Comparison Between Formalin and FineFIX, Number of Positive Results After PCR Amplification

Length (Bases)	Formalin Fixed	FineFIX
291	5/5	5/5
339	5/5	5/5
1000	0/5	5/5
1900	0/5	5/5
2400	0/5	5/5

RNA amplification by RT-PCR



Ten samples five from formalin-fixed and five from FineFIX-fixed tissues were subjected for RNA extraction.

500 ng of RNA extracted from all samples were subjected to DNase and RT-PCR amplification using a set of 170 bp (A), 200 bp (B), 600 bp (C) region of beta-actin gene.

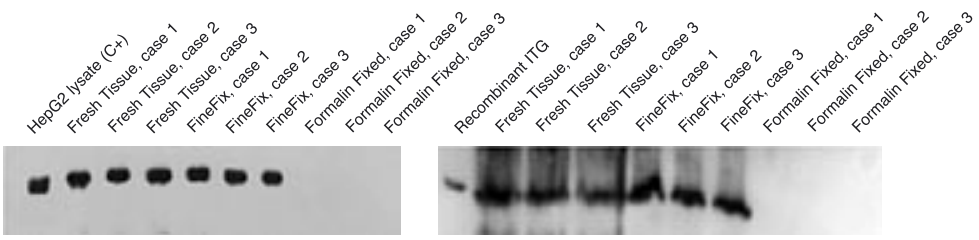
Each gel (A, B and C) shows:

Lane 1, molecular size marker; Lane 2-6, RNA obtained from formalin-fixed tissues; Lane 7-11, RNA obtained from FineFIX-fixed tissues; Lane 12, negative control; Lane 13, mRNA from HepG2 cells, positive control.

RNA Amplification Comparison Between Formalin and FineFIX, Number of Positive Results After RT-PCR Amplification

Length (Bases)	Formalin	FineFIX
77	5/5	5/5
170	5/5	5/5
200	0/5	5/5
450	0/5	5/5
600	0/5	3/5

Protein analysis - Western Blot



Seventy mg of whole protein extract both from formalin-fixed and from FineFIX - fixed tissues was loaded and run onto SDS - PAGE electrophoresis before blotting.

No proteins were detected from the formalin-fixed tissues either for alfa-tubulin (a-tubulin) and for tissue transglutamminase (tTG), although the extract from FineFIX-fixed tissues gave similar protein quality of those from fresh tissues.

FineFIX

The molecular fixative for

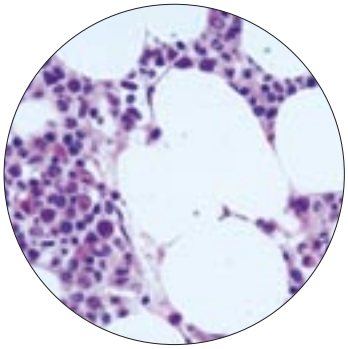
The advantages of ethanol-based fixatives

HOSPITAL TESTED FOR ROUTINE H&E, IHC, HC AND MOLECULAR ANALYSIS.

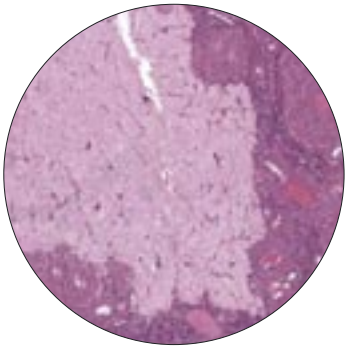
FineFIX has been extensively tested in a hospital laboratory environment to ensure its suitability staining for diagnostic purposes.
The morphology of FineFIX fixed tissues processed either conventionally or by microwaves is of a such high quality that it poses no difficulty for diagnosis.
Shrinkage and vacuolization are reduced to a fraction of what is experienced with traditional ethanol fixation. Pyknotic nuclei are also absent.

H&E STAINING

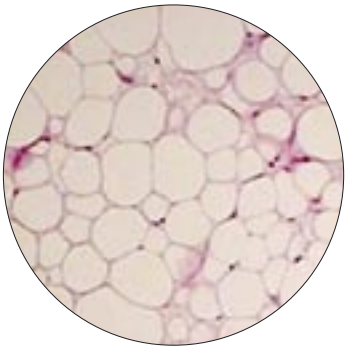
Tissues fixed in FineFIX show improved H&E staining intensity and cytological details.



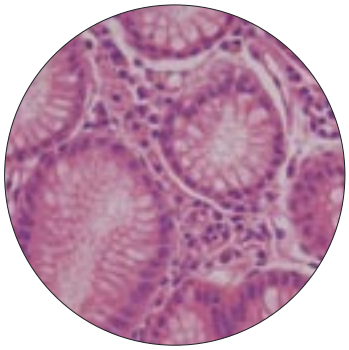
Bone marrow FineFIX H&E x400



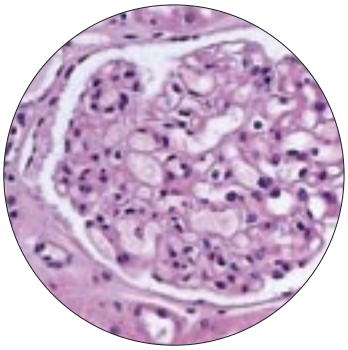
Ovarian Carcinoma. FineFIX H&E x 100



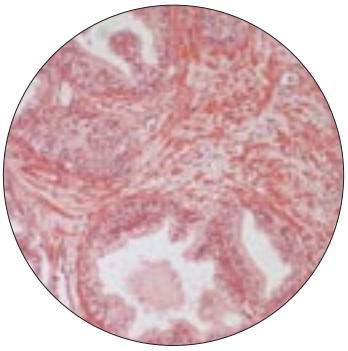
Adipous tumor. FineFIX H&E x 100



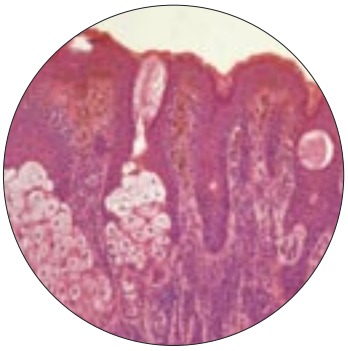
Gastric epithelium FineFIX H&E x400



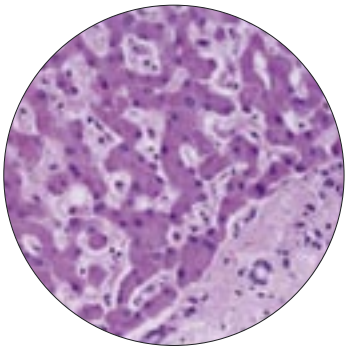
Kidney, FineFIX H&E x400



Prostate nodular hyperplasia FineFIX H&E x100



Skin melanocytic nevus FineFIX H&E x100



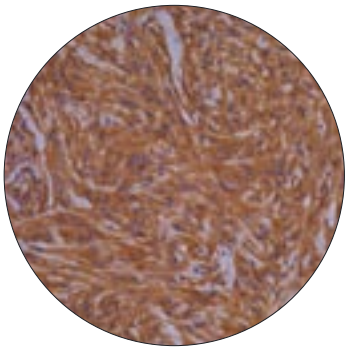
Liver. FineFIX H&E x 400

optimal DNA, RNA and protein preservation

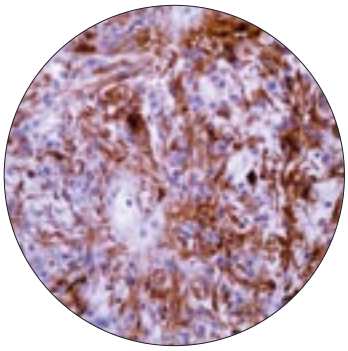
without their disadvantages

IMMUNOHISTOCHEMISTRY

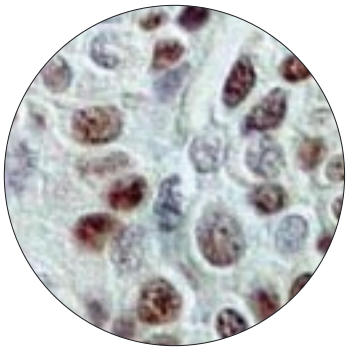
Improved Immunohistochemical staining by reducing the need for aggressive epitope retrieval.



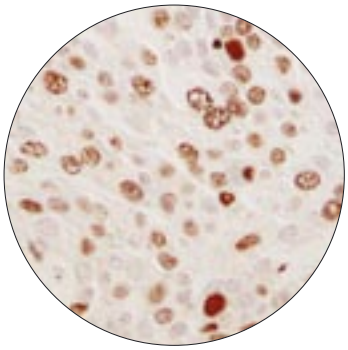
Meningioma staining for Vimentin x200



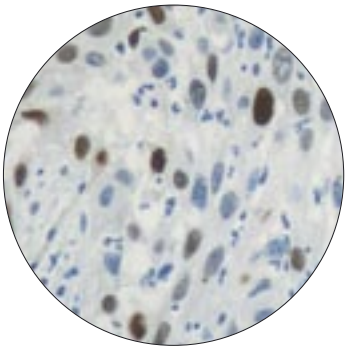
Ependymoma staining for Cytokeratin CAM5.2 x200



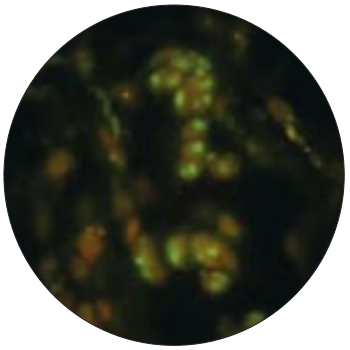
Breast. FineFIX ER (6F11)



Breast carcinoma staining for Mib-1 x400



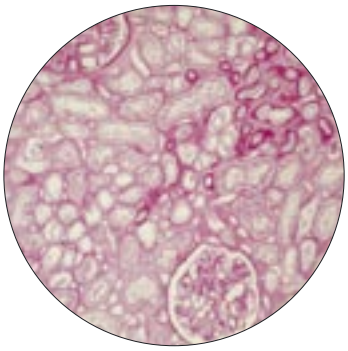
Breast carcinoma for P-53 x200



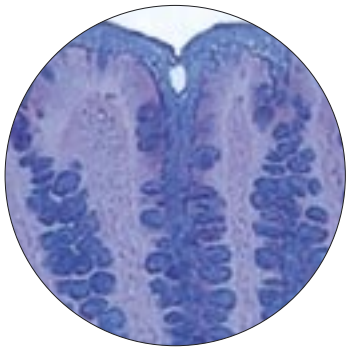
Breast. FineFIX Fish for Her2-neu

HISTOCHEMISTRY

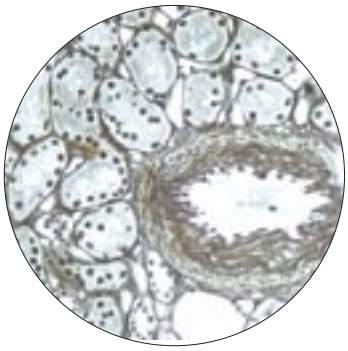
When using FineFIX, special stains procedures can be carried out without any change to existing protocols.



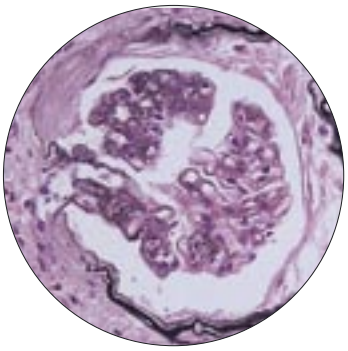
Kidney PAS stain FineFIX x100



Bowel, Alcian Blue. FineFIXx 100



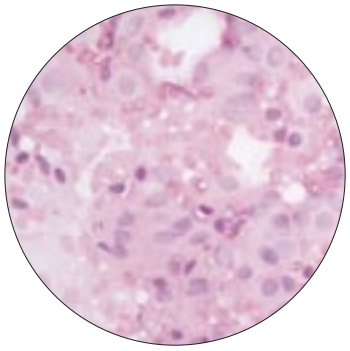
Kidney Reticulin stain FineFIX x200



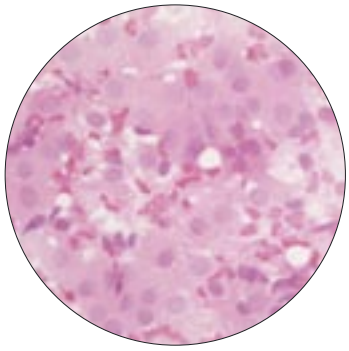
Kidney, Jones silver stain FineFIX x400

CYTOLOGY

FineFIX is well suited for use as a cytological fixative compared to ethanol.



Meningioma, FineFix x200



Meningioma, Ethanol x200

PERFUSION

Perfusion fixation in animal research laboratories has been carried out with optimum results.

MORE FineFIX ADVANTAGES

LOW TOXICITY

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.

Airborne Exposure Limits

FineFIX (working solution)	1000 ppm (TWA)*
Formalin, 10%	0.75 ppm (TWA)*

** Time waited average*

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

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 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.

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