Sigma-Aldrich®
Lab Materials & Supplies

Go Greener
Histology & Hematology

Sigma-Aldrich® is Committed to Bringing Safer Greener Alternatives to Our Customers.

- **Novec fluids** are non-flammable, low toxicity fluids for freezing histology samples.
- **Xylene Substitute** is non-toxic version of xylene which is compatible with all tissue
- **Formalin Free Tissue Fixatives** is a less toxic alternative to formalin. It is also compatible with a number of applications like PCR, IHC and *in-situ* hybridization
- **HistoChoice® Clearing Agent** is suitable as an alternative to toluene and xylene for dewaxing paraffin tissue sections
- **HistoChoice Tissue Fixative** is designed for molecular biology and used for *in-situ* hybridization

Cat. No.	Product Description
SHH0001-500ML	Novec™ 7000 Engineered Fluid
SHH0001-1L	Novec™ 7000 Engineered Fluid
SHH0002-500ML	Novec™ 7100 Engineered Fluid
SHH0002-1L	Novec™ 7100 Engineered Fluid
A5597-1GAL	Xylene Substitute
A5472-1GAL	Formalin Free Tissue Fixative
H2779-1L	HistoChoice® Clearing Agent
H2904-100ML	HistoChoice® Tissue Fixative
H2904-1L	HistoChoice® Tissue Fixative

Xylene Substitute

René J Buesa and Maxim V Peshkov

Annals of Diagnostic Pathology, 13(4), 246-256 (2009)

After the hazardous effects of xylene became indisputable in the 1970s, many potential substitutes became available, some with as many if not more hazards. This article discusses the inadequacy of 5 vegetable oils as substitutes, as well as the characteristics of 22 D-limonene-based substitutes, all less effective in their chemical role, some capable of inducing health problems, and costing more than twice as much as xylene. Some of the 35 alkane-based substitutes discussed are effective for tissue processing, less toxic, with a cost about the same as xylene, but are not very effective for dewaxing and other staining tasks. Isopropanol (2-propanol) alone or mixed with molten paraffin is a technically acceptable and cost-effective substitute for xylene for tissue processing, but in this study, we demonstrate that the best clearing agents from the sectioning quality and diagnostic value point of view, with automated or manual protocols, are mixtures of 5:1 and 2:1 isopropanol and mineral oil, followed by undiluted mineral oil, all at 50 degrees C, making them a safer and cheaper substitute than xylene. Using a 1.7% dishwasher soap aqueous solution at 90 degrees C to dewax before staining and oven drying the stained sections before coverslipping will eliminate xylene from the staining tasks. Tissue processors retorts and conduits can be dewaxed with a 2% solution of a strong glassware laboratory detergent. These 4 methodologies will make the histology laboratory xylene-free but, due to the natural resistance to change, many histotechs will be reluctant to adopt them if they think that their technical expertise could be jeopardized, and the only way these changes will succeed is if the pathologists, as stewards of the histology laboratory, commit to their implementation.

MilliporeSigma

Formalin Free Tissue Fixatives

M E Boon and L P Kok

Biotechnic and Histochemistry, 83(6), 261-277 (2008)

The German, F. Blum, introduced formalin as a fixative in 1893. Formalin rapidly became popular for hardening and preserving gross human and animal specimens. As a result, microscopy for diagnostic pathology by combining paraffin embedding and formalin fixation was developed. Alcohol-based fixatives have coagulation of proteins as their main preservative effect. Because there is no cross-linking, immunostaining is not compromised, and DNA and RNA is not damaged. Ethyl alcohol was used by Dutch scientists of the 18th century, but was replaced by the cheaper formalin. Addition of low molecular weight polyethylene glycol (PEG) optimized the coagulant fixative, Kryofix. The polyethylene glycol prevents excessive hardening and enhances the speed of coagulation of proteins. Kryofix was used on a large scale for skin biopsies in Leiden between 1987 and 2001. DNA preservation by the formulated coagulant fixative, BoonFix, is related to the concentration of ethyl alcohol, PEG and acetic acid. BoonFix has been used since 2004 in Leiden for over 40,000 diagnostic skin biopsies and more than 100,000 cervical samples. A literature review and three decades of experience with coagulant, formalin-free fixatives in pathology suggest that when health authorities realize that formalin invalidates expensive tests, it might eventually be eliminated legislatively from diagnostic pathology. Finally, coagulant fixation is optimal for microwave histoprocessing where ethyl alcohol is followed by isopropanol.

HistoChoice® Clearing Agent

1. Immunogenicity and contraceptive potential of three infertility-relevant zona pellucida 2 epitopes in the marsupial brushtail possum (Trichosurus vulpecula).

Janine A Duckworth et. al

Reproduction, 133 (1), 177-186 (2007)

In a previous study, three infertility-relevant epitopes of possum ZP2 (Pep12 (amino acids 111-125), Pep31 (amino acids 301-315), and Pep44 (amino acids 431-445)) were identified using sera from possums (Trichosurus vulpecula) immunized with recombinant possum zona pellucida 2 (ZP2) constructs, and a synthetic peptide library of possum ZP2 protein. In this study, the three peptides were conjugated to keyhole limpet hemocyanin and 300 mug of each conjugated peptide were administered subcutaneously to female possums (n = 20 per peptide) in complete Freund's adjuvant. Immunogen doses were repeated 3 and 6 weeks later using incomplete Freund's adjuvant. Control animals were immunized with either phosphate-buffered saline only (n = 10) or 300 mug keyhole limpet hemocyanin (n = 10), administered with the same adjuvants. Serum antibodies from animals immunized against these three epitopes bound to the corresponding possum ZP2 peptides, recombinant possum ZP2 protein constructs, and native zona. Possum fertility was assessed following superovulation and artificial insemination. Peptides Pep12 and Pep31 had no significant effects on fertility parameters ($P > 0.05$). However, animals immunized with Pep44 had lower egg fertilization rates (immunized 19.5% versus control 60.5%, $P < 0.05$) and produced significantly fewer embryos than control animals (immunized 0.5 embryos versus control 2.4 embryos, $P < 0.05$). The number of Pep44-immunized females that produced embryos was reduced by 64%. Identification and characterization of possum infertility-relevant epitopes on possum ZP2 protein will assist development of safe, humane, and possum-specific immunocontraceptive vaccines for controlling the introduced possums in New Zealand.

2. A simplified method for combined immunohistochemistry and *in-situ* hybridization in fresh-frozen, cryocut mouse brain sections.

Sathyanesan Samuel Newton et. al

Brain Research Protocols, 9 (3), 214-219 (2002)

A method is described to perform combined immunohistochemistry and in situ hybridization in mouse brain sections. The protocol is specific to sections mounted on glass slides. In contrast to earlier methods that require either paraffin embedding or perfusion of the brain with paraformaldehyde, this protocol can be carried out on fresh-frozen, cryostat cut post-fixed sections. This simple and concise protocol increases the applicability of the technique as the RNAse-free immunodetection of antigen is useful by itself for immunologically identifying specific cells of interest and then examining gene expression in those cells using techniques such as real-time PCR and microarray analysis. The use of fresh-frozen, cryocut sections enables reliable detection of easily perturbable post-translational modifications such as phosphorylation and improves the quality of results obtained in subsequent in situ hybridization by reducing the background signal and interference from lower cell layers. Inducible transgenic mice that express either a dominant negative mutant form of the cAMP response element binding protein (mCREB) or CREB, in discrete brain regions, were used in this study. The combined immunohistochemistry and in situ hybridization protocol was used to examine colocalization of enkephalin or dynorphin mRNA, both downstream targets of CREB-mediated gene expression, in cells expressing transgenic mCREB or CREB.

HistoChoice Tissue Fixative

J Melrose et. al

Biotechnic and Histochemistry, 83(1), 47-53 (2008)

Histochoice is a proprietary nontoxic, non-cross-linking fixative designed by the manufacturer to replace formaldehyde based fixation protocols. We compared Histochoice and formalin fixation for several cartilaginous tissues including, articular and growth plate cartilage, meniscus and intervertebral disc. The tissues were stained with general histology stains including toluidine blue for tissue proteoglycans, picrosirius red to evaluate collagenous organization, and hematoxylin and eosin to assess cell morphology. The chondroitin sulfate and heparin sulfate substituted proteoglycans aggrecan and perlecan were also immunolocalized in some of the tissues to provide a comparison. Histochoice did not fix deep into the tissue blocks resulting in focal loss of aggrecan and other matrix components from the more central regions of the blocks. This was evident in toluidine blue stained sections of immature tibial articular cartilage where loss of glycosaminoglycan was significant in Histochoice fixed tissues. Histochoice fixation worked well, however, in the aggrecan and perlecan immunohistology applications where its non-cross-linking traits were conducive to epitope retrieval and identification by primary antibodies to extracellular matrix components.

To place an order or receive technical assistance

Order/Customer Service: **SigmaAldrich.com/order**
Technical Service: **SigmaAldrich.com/techservice**
Safety-related Information: **SigmaAldrich.com/safetycenter**

SigmaAldrich.com

Lit. No. sh8707
2017 - 06723
09/2017