

## Product Information

### Formalin Free Fixative, Accustain™

Catalog Number **A5472**  
Store at Room Temperature

#### Product Description

Formaldehyde (Formalin) and formulations containing it such as 10% Neutral Buffered Formalin (NBF) are commonly used to preserve tissues for subsequent staining and analysis. However, due to the intrinsic hazardousness of formaldehyde, non-formalin based fixative reagents may be preferred as safer alternatives and to reduce nucleic acid damage within the tissue that occurs with formalin fixing for subsequent PCR and other genomic techniques.<sup>1</sup>

Formalin Free Fixative, Accustain is a tissue fixative that is a less toxic alternative to formalin. It can replace the commonly used 10% neutral buffered formalin solution for routine tissue procedures and performs well with PCR, *in-situ* hybridization, and immunohistological staining. The reagent is a proprietary alcohol-based solution with hydroxylated compounds.

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

#### Preparation Instructions

Formalin Free Fixative, Accustain is supplied ready-to-use.

#### Storage/Stability

Store the product at room temperature.

#### Procedure

Different fixatives will have different rates of tissue penetration and fixation. Formalin fixation is the most commonly used method of preserving tissues and is the reagent against which all other fixatives are compared.

When first using Formalin Free Fixative, Accustain, Sigma recommends performing a comparison study by taking duplicate tissue specimens and fixing in both 10% Neutral Buffered Formalin and Formalin Free Fixative, Accustain.

1. Fixation should begin as soon as possible after tissue sampling.
2. Be sure the tissue is placed in the proper fixative. If the tissue cannot be immediately placed into the fixative, keep the tissue moist and cool. Typically the tissue is kept moist with normal saline or isotonic PBS.
3. The ideal ratio of fixative to tissue should be in the range of 20-50 parts of fixative to 1 part tissue. The ratio of fixative to tissue should never be less than 10-20 parts of fixative to 1 part tissue.
4. Whole organs should be injected with fixative as well as immersed in fixative. Large organs can be sliced to allow better penetration of the fixative into the tissue.
5. Hollow organs can be injected with fixative or can be packed with absorbent cotton soaked in fixative before immersion. Some organs such as colon may be opened and pinned to a corkboard before immersion in the fixative.
6. The time needed for fixation can range from just a few hours to several weeks. The time needed will vary upon the tissue type and the size or thickness of the specimen.
7. After fixation has been completed, the fixed tissue should be trimmed to no more than 3 to 5 mm in thickness and placed on a tissue processor for paraffin processing.

Cells may be fixed for 20 minutes and then washed with phosphate buffered saline (PBS) prior to subsequent processing for immunostaining.<sup>2,3</sup>

Formalin Free Fixative, Accustain has been used with human umbilical cord blood endothelial colony-forming cells (ECFC)<sup>4</sup> and to fix cell cultures prior to DAPI counterstaining and immunocytochemistry.<sup>5</sup>

Murine brains were fixed by immersion in Formalin Free Fixative, Accustain for at least 12 hours at 4 °C after euthanization.<sup>6</sup>

A product similar to Formalin Free Fixative, Accustain was found to be a suitable alternative to 4% paraformaldehyde solution (MEMPFA) tissue fixation with *Xenopus laevis* embryos for immunohistochemistry.<sup>7</sup>

## References

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3. Yee, D., et al., Hyaluronic acid hydrogels support cord-like structures from endothelial colony-forming cells. *Tissue Eng. Part A*, **17**, 1351-61 (2011).
4. Hanjaya-Putra, D. et al., Controlled activation of morphogenesis to generate a functional human microvasculature in a synthetic matrix. *Blood*, **118**, 804-15 (2011).
5. Freudenberg, U., et al., A star-PEG-heparin hydrogel platform to aid cell replacement therapies for neurodegenerative diseases. *Biomaterials*, **30**, 5049-60 (2009).
6. Hlavaty, J., et al., Comparative evaluation of preclinical *in vivo* models for the assessment of replicating retroviral vectors for the treatment of glioblastoma, *J. Neurooncol.*, **102**, 59-69 (2011).
7. Acton, A., et al., An examination of non-formalin-based fixation methods for *Xenopus* embryos. *Dev. Dyn.*, **233**, 1464-9 (2005).

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