

GAF® - INSTRUCTIONS

HOW TO USE GAF® (GLYOXAL ACID FREE FIXATIVE)



GAF® is a histological fixative alternative to Formalin based on a buffered aqueous solution of Glyoxal (Oxalic Dialdehyde). The acids contained in the commercial glyoxal solution (mainly Glyoxylic and Glycolic acids) have been removed (with ion exchange resins) and the pH is kept at around 7.2-7.4, as evidenced by the pink colour (due to traces of Phenol Red as an indicator). GAF® fixative is protected by an international patent, is CE labelled and FDA approved and is produced by ADDAX Biosciences S.r.l.

GAF® acts with an aldehydic reaction mechanism. Therefore, the method of reaction and tissue fixation are analogous to those of Formaldehyde. However, not being volatile, GAF® does not have the toxic, allergenic and carcinogenic characteristics linked to the formation of Formaldehyde vapours.

GAF® Fixative is stable for one year when stored in a refrigerator between 2 °C and 8 °C. It can be stored at room temperature for at least one month: after that, it becomes progressively more acidic, as shown by the indicator colour turning yellow. In this case, it is recommended not to use the reagent.

INTENDED USE

GAF® (Glyoxal Acid-Free) Fixative is an in vitro diagnostic device used as histological fixative alternative to Formalin.

DEVICE DESCRIPTION

GAF® (Glyoxal Acid-Free) Fixative is a water solution of Glyoxal, acid-free, in a Phosphate Buffer pH 7.1-7.8. The in-vitro diagnostic device is intended for histological fixation only.

STABILITY

GAF® (Glyoxal Acid-Free) Fixative is stable (pink colour, pH ≥ 7) for 12 (twelve) months when stored between 2 °C and 8 °C.

The device can be kept at room temperature for at least 30 (thirty) days.

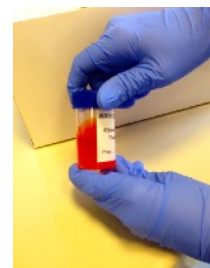
Do not use if the colour changes to yellow (acidified).

GAF® (Glyoxal Acid Free) Fixative must be use according to the instructions below

Analogous to Formalin, the following general rules and standards of good practice must be applied:

- Fix as soon as possible: remember that degeneration starts as soon as the cells are deprived of blood supply.
- If fixation is not immediately possible, refrigerate but do not freeze. Freezing the tissue will result in considerable damage due to the formation of ice crystals.
- Do not allow specimens to dry: drying of specimen surfaces will cause permanent damage.
- Cavities should be opened: If possible, hollow organs or samples with natural cavities should be opened to allow immediate access of the fixative.
- Adequate volume of fixative is vital (20:1 ratio between fixative and material at least): the specimen must be completely immersed in an amount of fixative containing sufficient effective components to fix, since these components are depleted during the fixation reactions.
- Room temperature: Initial fixation is preferable at room temperature (18-22° C).
- The fixative should only be used once.

1. Open the cap of GAF® (Glyoxal Acid-Free) Fixative ensuring that the container is held in vertical position.



2. Remove completely the cap of GAF® (Glyoxal Acid-Free) Fixative paying attention that the container remains in vertical position.



3. Insert the specimen into GAF®. If needed, gently shake the needle / forceps in order to release the specimen. Check if the specimen is completely immersed into the fixative.



4. Tighten the cap on the container firmly.



FIXATION TIME AND METHODS

- The fixation times and procedures are similar to those currently used for Formalin ("Neutral Buffered Formalin" or "NBF"):
- **small histological specimens** (*core biopsies and outpatient samples, less than 5mm in size*): about 6 hours. The tissue is immersed in the vials' liquid, then transported at room temperature to the pathology laboratory, where they are subsequently transferred in ethyl alcohol for paraffin embedding;
- **larger histological specimens** (such as *surgical samples*): longer times (24-48 hours) are recommended, as per the current practice for Formalin fixation. Volumetric ratio should be kept adequate (at least 20:1).

SAFETY

GAF® (Glyoxal Acid-Free) Fixative is non-carcinogenic and non-volatile as it has a very low vapour pressure and therefore cannot evaporate to any significant extent. GAF® (Glyoxal Acid-Free) has a significantly less severe toxicological impact than formalin. However, the use of nitrile gloves, protective goggles and personal protective equipment required by local safety protocols is recommended.

Vapour monitoring is not required. Furthermore, regulatory / normative compliance issues applicable to formalin (related to carcinogenicity and inhalation exposure) do not apply to GAF®.

IMMUNOHISTOCHEMISTRY, FISH AND MOLECULAR BIOLOGY.

Analogous to what is currently practised on histological sections of Formalin-fixed tissues, immunohistochemical reactions for antigens of diagnostic interest can be performed on GAF-fixed tissues using current equipment (Ventana, Leica etc.) and following antigen retrieval and staining protocols specific for the various antigens. Although most antigens require the same protocols as those recommended for Formalin-fixed tissues, slight variations are recommended in some cases. Optimized protocols for the most commonly investigated antigens can be found at www.addaxbio.com under 'Product/IHC validation protocols'.

FISH reactions can be performed on GAF-fixed tissues according to current methods. A slight blue background fluorescence detectable in nuclei is removed by a brief treatment with TRIS-HCl buffer pH 8.6 (see Bussolati et al., Plos One 12:e0182965; 2017, downloadable at www.addaxbio.com).

Nucleic acids (DNA and RNA) are preserved in GAF-fixed tissues, and can be extracted and analysed according to methods and procedures currently used in buffered Formalin-fixed tissues (see Plos One 12:e0182965; 2017). Quantity and quality of nucleic acids obtained from GAF-fixed tissues are analogous to those currently obtained from Formalin-fixed tissues.

VALIDATION

Performance of GAF® as a histological fixative, as compared to NBF, was tested in Trials proving its diagnostic value. The full data of the ADDAX-GAF 2019 Validation Trial can be accessed at: https://addaxbio.com/wp-content/uploads/2022/05/ADDAX-GAF-2019_CPSR_V1.0_17Feb2022_fully-signed_compressed.pdf

DISPOSAL

GAF® (Glyoxal Acid-Free) Fixative does not contain any hazardous substances and does not possess any characteristics that would qualify it as hazardous according to EPA (Environmental Protection Agency) standards. Ethanol is present in the product as a non-flammable solution.

GAF® (Glyoxal Acid-Free) Fixative must be disposed of as a chemical substance. After use, it must be disposed of as a special hospital biological waste in accordance with the regulations in force.

WARNINGS AND PRECAUTIONS

GAF® (Glyoxal Acid-Free) Fixative is not a general formalin substitute: do not use for purposes other than histological fixation.

The product is intended for use by specialized technical personnel.

Keep container tightly closed.

Do not use the product if the container is damaged.

Store the product and keep it according to the instructions provided.

PRECAUTIONARY STATEMENTS (GHS)

P201: Obtain special instructions before use.

P210: Keep away from heat/sparks/open flames/hot surfaces — No smoking during use and while close to the product.

P262: Avoid contact with eyes, skin, or on clothing.

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P303+P305+P353+P361+P308+P310: In case of contact with the skin (or hair) or with eyes: take off immediately all contaminated clothing, rinse the skin and all surface that got in contact with the product with specific detergents. In case of ingestion or possible ingestion: Immediately call a poison centre or a Doctor.

HAZARD STATEMENTS (GHS)

H302: Harmful if swallowed.

H315: Causes skin irritation.

H317: May cause an allergic skin reaction.

H319: Causes serious eye irritation.

H341: Suspected of causing genetic defects.

DEVICE COMPOSITION

Component	CAS	CE	INDEX	CONTENT (%)
GLYOXAL	107-22-2	203-474-9	605-016-00-7	1-6%
ETHANOL	64-17-5	200-578-6	603-002-00-5	3-6%
PROPYLENE GLYCOL	57-55-6	200-338-0		3-6%
RED PHENOL	143-74-8	143-74-8	-	<0.002%
PHOSPHATE BUFFER	-	-	-	10-12%
WATER	-	-	-	At volume

SYMBOL GLOSSARY

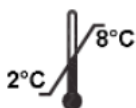
CE mark



In-Vitro Diagnostic Device



Manufacturer



Storage conditions: Store between 2°C and 8 °C



Batch code



Use by date - Symbol followed by expiry date expressed in YYYY/MM



Consult instructions for use



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