

## GAF<sup>®</sup> Fixative - Glyoxal Acid Free

# Technical Data Sheet and Instructions for Use

GAF<sup>®</sup> Fixative (Glyoxal Acid Free) is an histological fixative alternative to Formalin based on a buffered aqueous solution of Glyoxal.

The acids contained in the commercial Glyoxal solution have been removed through a patented deprivation process and the pH is maintained at around 7.1 - 7.8, as evidenced by the pink colour (pH indicator).

The GAF<sup>®</sup> fixative is protected by an international patent and is produced by ADDAX Biosciences S.r.l.

GAF<sup>®</sup> acts with an aldehydic reaction mechanism. Therefore, the method of reaction and tissue fixation are analogous to those of Formaldehyde. However, being non-volatile, GAF<sup>®</sup> is free of the toxic, allergenic and carcinogenic characteristics associated with Formaldehyde vapours.

GAF<sup>®</sup> fixative is stable for twenty-four months if stored in a refrigerator between 2 °C and 8 °C. It can be stored at room temperature (5 °C - 25 °C) for at least three months. In any case, it can always be used if the colour is pink. If the colour turns yellow, it is recommended not to use the reagent.

### INTENDED USE

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

### DEVICE DESCRIPTION

GAF<sup>®</sup> Fixative (Glyoxal Acid Free) is an aqueous solution based on acid-free glyoxal, in a phosphate buffered solution, with a pH of 7.1-7.8.

The in vitro diagnostic device is intended exclusively for histological fixation.

### STABILITY – STORAGE INSTRUCTIONS

GAF<sup>®</sup> Fixative (Glyoxal Acid-Free) is stable (pinkish colour, pH  $\geq$  7) for 24 (twenty-four) months when stored between 2°C and 8 °C.

Before use, keep the product at room temperature to allow the dissolution of any crystals formed due to excessively low temperatures, and perform the fixation with the product at room temperature.

The device can be stored at room temperature (5 °C - 25 °C) for at least 90 (ninety) days.

Do not use it in case of acidification (colour change to yellow).

**GAF Fixative® (Glyoxal Acid Free) must be used in accordance with the instructions for use below**

As per Formalin, the following general rules and standards of good practice apply for the treatment of histological tissues:

- Fix as early as possible: remember that degeneration begins as soon as the cells are deprived of a blood supply.
- If fixation is not immediately possible, refrigerate but do not freeze. The freezing of the tissue will result in considerable damage due to the formation of ice crystals.
- Do not allow samples to dry out: The drying of the sample surfaces will cause permanent damage.
- Cavities must be opened if possible, hollow organs or samples with natural cavities must be opened to allow the immediate access of the fixative.
- Pay attention to the volume of fixative: an adequate ratio between fixative and fixed material (at least 20:1) is crucial. The sample must be completely immersed in a quantity of fixative containing sufficient components for fixation, since they are naturally exhausting as part of the fixation reaction.
- It is preferable that the initial fixation takes place at room temperature (18-22° C).
- The fixative must only be used once

1. Opening the lid of GAF® making sure to hold the container in a vertical position.



2. Completely remove the GAF® lid taking care that the container remains in an vertical position.



3. Place the sample in the GAF. If necessary, gently shake the needle or forceps to release the sample. Check that the sample is fully immersed in to the fixative



4. Close the lid firmly lid by screwing it firmly tight.



## TIMING AND METHOD OF FIXATION

The timing and method of fixation are similar to those currently used for Neutral Buffered Formalin ('NBF'):

**for small pieces** (core biopsies and outpatient samples, size less than 5 mm), the fixation time is about 6 hours. The tissue is immersed in the liquid present in the flacons. They are then transferred, at room temperature, to the analysis laboratory (Pathological Anatomy), where they are subsequently passed in Ethyl Alcohol for paraffin embedding.

**for more voluminous histological pieces** (such as surgical samples), longer fixation times (approx. 24-48 hours) are recommended, similar to the current practice for Formalin fixation. Volumetric ratio should be kept adequate (at least 20:1).

Unlike formalin fixation, GAF® fixation does not harden the tissue, keeping its colour and consistency similar to the pre-fixed 'fresh' condition.

## ALIQUOTATION

The product is also available in bulk format, designed to allow flexible volume management according to specific laboratory needs. The bulk format allows aliquoting to smaller capacity containers, ensuring accurate distribution for customised uses.

## SAFETY

GAF® (Glyoxal Acid-Free) Fixative is not carcinogenic, is not volatile (as it has a very low vapour pressure; therefore, it cannot evaporate to a significant extent), and consequently has a much less severe toxicological impact than Formalin.

However, it is advisable to handle the product using protective gloves, goggles and personal protective equipment required by local safety protocols.

Vapour monitoring is not required.

In addition, regulatory compliance issues related to Formalin (related to carcinogenicity and inhalation exposure) do not exist.

## IMMUNOHISTOCHEMISTRY AND MOLECULAR BIOLOGY

In analogy to the current practice on histological sections of Formalin-fixed tissues, immunohistochemical reactions for antigens of diagnostic interest can be performed on GAF®-fixed tissues using current equipment (e.g. Ventana, Leica, etc.) and following specific antigen retrieval and staining protocols for the various antigens. Although most antigens require **the same protocols as those recommended** for Formalin-fixed tissues, slight variations are advisable in some cases.

At [www.addaxbio.com](http://www.addaxbio.com), under 'Product/IHC validation protocols', you can find recommended optimal protocols for the most commonly investigated antigens are available.

Nucleic acids (DNA and RNA) are preserved in tissues fixed in GAF, and can be extracted and analysed according to methods and procedures currently used in tissues fixed in buffered Formalin (cf. Bussolati et al.; Plos One 12:e0182965; 2017, downloadable at [www.addaxbio.com](http://www.addaxbio.com)).

The quality of nucleic acids obtained from GAF® fixed tissues are better to those currently obtained from FFPE (cf. Berrino et al.; Laboratory Investigation, 2024, downloadable at [www.addaxbio.com](http://www.addaxbio.com))

## FISH

FISH reactions are performed on tissues fixed in GAF® with the standard procedure.

A slight background blue fluorescence detectable in the nuclei is removed by a brief treatment (following deparaffinization of the slide) with TRIS-HCl Buffer pH 8.6 (1) for a variable time ranging from 10 to 30 minutes..

As reported in guidelines and scientific publications, (5)(6) (7) (8) in buffered formalin-fixed tissues, the timing of fixation has a significant influence on the quality of in situ hybridisation by recommending variable fixation times (6h - 72h) and indicating 24h as the optimal time. These recommendations can also be applied for tissues fixed in GAF.

PLEASE NOTE: If treatment with alkaline buffer is not sufficient to completely remove background fluorescence, a shorter digestion time with peptidase enzyme (as suggested by some probe companies (2) can be used.

Bussolati G, Annaratone L, Berrino E, Miglio U, Panero M, Cupo M, Gugliotta P, Venesio T, Sapino A, Marchiò C. Acid-free glyoxal as a substitute of formalin for structural and molecular preservation in tissue samples. *PLoS One*. 2017 Aug 10;12(8):e0182965. doi: 10.1371/journal.pone.0182965. PMID: 28796828; PMCID: PMC5552132 (scaricabile sul sito [www.addaxbio.com](http://www.addaxbio.com))

(1) ZytoLight FISH tissue implementation ([https://www.zytovision.com/downloads\\_products/manuals/it/z-2028-ce-ivd-it.pdf](https://www.zytovision.com/downloads_products/manuals/it/z-2028-ce-ivd-it.pdf))

(3) Broude NE, Budowsky EI. The reaction of glyoxal with nucleic acid components. 3. Kinetics of the reaction with monomers. *Biochim Biophys Acta*. 1971 Dec 30;254(3):380-8. doi: 10.1016/0005-2787(71)90868-9. PMID: 5137601.

(4) Nakaya K, Takenaka O, Horinishi H, Shibata K. Reactions of glyoxal with nucleic acids. Nucleotides and their component bases. *Biochim Biophys Acta*. 1968 Jun 18;161(1):23-31. doi: 10.1016/0005-2787(68)90290-6. PMID: 5690799.

(5) Kondo J, Yoshino S, Iida M, Takeda S, Nakashima C, Watanabe Y, Nishiyama M, Tokumitsu Y, Shindo Y, Nishimura T, Suzuki N, Hoshii Y, Itoh H, Nagano H. Effects of Extended Fixation on Advanced Gastric Cancer HER2 Status Assessment Using IHC and FISH. *Anticancer Res*. 2024 Feb;44(2):621-630. doi: 10.21873/anticancer.16851. PMID: 38307565.

(6) Selvarajan S, Bay B-H, Choo A, et al. Effect of Fixation Period on HER2/neu Gene Amplification Detected by Fluorescence In Situ Hybridization in Invasive Breast Carcinoma. *Journal of Histochemistry & Cytochemistry*. 2002;50(12):1693-1696. doi:10.1177/002215540205001215

(7) [https://sigu.net/wp-content/uploads/2020/11/raccomandazioni\\_per\\_lanalisi\\_fish\\_interfasica\\_su\\_sezioni\\_istologiche\\_in\\_ambito\\_oncologico.pdf](https://sigu.net/wp-content/uploads/2020/11/raccomandazioni_per_lanalisi_fish_interfasica_su_sezioni_istologiche_in_ambito_oncologico.pdf)

(8) <https://documents.cap.org/documents/cap-14-formalin-fixation-time-2019.pdf>

## WASTE DISPOSAL

GAF® (Glyoxal Acid-Free) Fixative does not contain any hazardous substances and has no characteristics that qualify it as environmentally hazardous. It does contain ethanol, but as a non-flammable solution.

The product must be disposed of as a chemical substance in accordance with current regulations. After use (contact with tissue) it should be disposed of as hospital biological waste in accordance with current regulations.

## CAUTIONARY ADVICE (GHS)

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be worn outside the workplace.

P280 Wear protective gloves.

P302+P352 IF ON SKIN: Wash with plenty of water.

P333+P313 IF skin irritation or rash occurs: Get medical advice.

P501 Dispose of contents/container in accordance with local regulations.

## HAZARD STATEMENTS (GHS)

H317: May cause allergic skin reaction.

## WARNINGS AND PRECAUTIONS

General Formalin substitute: do not use for purposes other than histological fixation.

The product is intended for use by specialized technical personnel.

Keep the container tightly closed.

Do not use the product if the container is damaged.

Store the product correctly.

Do not reuse.

Do not use the product if it has a yellowish colour (and is therefore acidified).

The user must report any serious incident occurring in connection with the device to Addax Biosciences and to the competent authority.

### COMPOSITION

quantity	name	identification number	classification	registration number
≥1-<2.5%	Gliossale	CAS:107-22-2 EC:203-474-9 Index:605-016-00-7	Muta. 2, H341; Acute Tox. 4, H332; Eye Irrit. 2, H319; Skin Irrit. 2, H315; Skin Sens. 1, H317	01-2119461733-37-XXXX

### LEGEND



CE mark



In vitro diagnostic medical device



Manufacturer



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



Lot number



Expiring date - expressed in YYYY/MM



Consult the Instructions for Use

## AVAILABLE SIZES

REF	Type	name	description	Reg. progressive n°	CND
<b>GAF000010P40</b>	PREFILLED	GAF® fixative 10 ml	PP container with screw cap pre-filled with 10ml GAF on a total volume of <b>20ml</b>	2565131/R	W01030705
<b>GAF000025P40</b>	PREFILLED	GAF® fixative 25 ml	PP container with screw cap pre-filled with 25ml GAF on a total volume of <b>60ml</b>	2565144/R	W01030705
<b>GAF000090P12</b>	PREFILLED	GAF® fixative 90 ml	PP container with screw cap pre-filled with 90ml GAF on a total volume of <b>160ml</b>	2565215/R	W01030705
<b>GAF000090P24</b>	PREFILLED	GAF® fixative 90 ml	PP container with screw cap pre-filled with 90ml GAF on a total volume of <b>160ml</b>	2565217/R	W01030705
<b>GAF000125P24</b>	PREFILLED	GAF® fixative 125 ml	PP container with screw cap pre-filled with 125ml GAF on a total volume of <b>250ml</b>	2565224/R	W01030705
<b>GAF000250P12</b>	PREFILLED	GAF® fixative 250 ml	PP container with screw cap pre-filled with 250ml GAF on a total volume of <b>500ml</b>	2565226/R	W01030705
<b>GAF000250B8</b>	BULK	GAF® fixative 250 ml	250ml bottle - PET	2565228/R	W01030705
<b>GAF001000B1</b>	BULK	GAF® fixative 1000 ml	1 litre bottle -PET	2565230/R	W01030705
<b>GAF003000B1</b>	BULK	GAF® fixative 3 l	3 litre jerry can - HDPE	2565231/R	W01030705
<b>GAF005000B1</b>	BULK	GAF® fixative 5 l	5 litre jerry can - HDPE	2565234/R	W01030705
<b>GAF010000B1</b>	BULK	GAF® fixative 10 l	10 litre jerry can - HDPE	2565236/R	W01030705



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