DATA SHEET
G.A.F.

1) Product codes

ADDAX Glyoxal A.F. Fixative 1000 ml
ADDAX Glyoxal A.F. Fixative vial (25 ml)

2) Unit of sell

ADDAX Glyoxal A.F. Fixative vial (25ml) in 50/100 pieces format

Use

Glyoxal Acid-Free (G.A.F.) Fixative has a penetration rate into tissues similar to that of formalin, and will produce excellent results in a conveniently short time. Small biopsies will fix in 1-2 hours but can be left in the fixative for longer time without any appreciable detrimental effect. General surgical specimens not thicker than 4 mm will fix adequately in 6-24 hours at room temperature. Allow longer time when fixing gross specimens. No shrinking artefacts. Basement membranes and cell membranes are clearly defined. Nuclear patterns and cytological details are good. Notably (at variance with what observed when using acidic Glyoxal preparations), erythrocytes and eosinophyls are well preserved.

Immunohistochemistry

IHC was employed to assess antigen preservation. The selected target proteins represented a broad spectrum of proteins distributed in different subcellular compartments (cytoplasm, cell membrane, nucleus). No discrepancies in subcellular localization of protein expression were observed in the differently fixed samples (Figure 2). Nuclear antigens required an optimization of the antigen retrieval procedure, i.e. longer duration of the antigen retrieval (60/90 min *versus* 30 min, Table 1). Following optimization, all nuclear antigen except Ki67 gave results super-imposable to the reactions performed on PBF fixed samples. Ki67 expression was observed to be less pervasive in GAF fixed samples compared to corresponding PBF samples. A 60 min long antigen retrieval procedure for Ki67 reaction gave better results, however Ki67 indices were lower (mean: 6%) than in formalin fixed samples here analyzed.
For nuclear antigens we also tested antigen retrieval treatment at high temperature (125°C) in a highly basic buffer (pH 8.6). Such treatment did not yield significant improvements compared to longer duration of standard antigen retrieval procedures.
For all of the remaining cytoplasmic and membrane markers so far tested, time of antigen retrieval was the same as for standard FFPE tissues.

Antibodies and antigen retrieval methods used for immunohistochemical reactions. Ab: antibody; CC1/CC2: cell conditioning Ventana Apparatus; CEA: carcinoembryonic antigen; CGA: chromogranin; CK: cytokeratin; F: formalin fixation; G: glyoxal fixation; SMA: smooth muscle actin; CAD-E: E-cadherin; PHH3: phospho-histone H3; TTF1: thyroid transcription factor 1.

Antibody	Clone	Species	Manufacturer	Dilution	Antigen Retrieval	Primary Ab Incubation
HER2	4b5	Rabbit	Roche	Prediluted	CC1, 36min	20min
Ki67	MIB-1	Mouse	Dako	1:50	CC1, 36min (F) CC2, 60min (G)	20min
CK20	SP33	Rabbit	Roche	Prediluted	CC1, 36min	20min
PanCK	AE1/AE3/PCK26	Mouse	Roche	Prediluted	Protease 1, 4min	20min
SMA	1A4	Mouse	Roche	Prediluted	CC1, 8min	20min
CEA	TF 3H8-1	Mouse	Roche	Prediluted	CC1, 8min	20min
S100	polyclonal	Rabbit	Roche	Prediluted	No treatment	20min
CGA	LK2H10	Mouse	Roche	Prediluted	No treatment	20min
CDX2	EPR2764Y	Rabbit	Roche	Prediluted	CC1, 60min (F) CC1, 92min (G)	20min
TTF1	8G7G3/1	Rabbit	Roche	Prediluted	CC1, 36min (F) CC1, 90min (G)	24min
PHH3	polyclonal	Rabbit	Roche	Prediluted	CC1, 36min (F) CC1, 60min (G)	32min
CAD-E	EP700Y	Mouse	Roche	Prediluted	Protease 1, 4min (F) CC1, 60min (G)	32min
CD3	2GV6	Rabbit	Roche	Prediluted	CC1, 36min	20min
CD20	L26	Mouse	Roche	Prediluted	CC1, 20min	20min

Nucleic Acids

At variance with what observed when Acidic Glyoxal preparations are used, Glyoxal A.F. fixative provides an excellent preservation of Nucleic acids (DNA and RNA), fully comparable to that obtained in tissues fixed in buffered Formalin. We even observed a significant enrichment of longer DNA fragment size in GAF-fixed compared to PBF-fixed samples. (**see:** Gianni Bussolati, Laura Annaratone, Enrico Berrino, Umberto Miglio, Mara Panero, Marco Cupo, Patrizia Gugliotta, Tiziana Venesio, Anna Sapino, Caterina Marchiò, Acid-free glyoxal as a substitute of formalin for structural and molecular preservation in tissue samples. PLoS ONE 12(8): e0182965).

In FISH analysis, results were super-imposable to those obtained in PBF-fixed tissues. The mild autofluorescent background with FISH testing is likely due to the link of glyoxal to the DNA bases, mainly to guanine and cytidine, which results in cross-links most likely responsible of the observed nuclear auto-fluorescence. The autofluorescence disappeared following a short passage in an alkaline buffer pH 8.6
Tests performed with the three sequencing platforms (Sanger sequencing, Pyrosequencing, Sequenom, MassARRAY®) gave comparable results for *KRAS* testing on both PBF- and GAF-fixed samples suggesting suggest a DNA/RNA fragmentation not lower than that induced by formalin crosslinking. Of note, an enrichment of the GAF-fixed samples for longer DNA fragment size was observed (see Bussolati et al., 2017).

Mode of action

The active ingredient is glyoxal, in a buffered mixture of water and alcohols. The content in ethanol and glycol is minimal and does not contribute directly to fixative effects. Glyoxal is a di-aldehyde, structured as if two formaldehyde molecules were attached back-to-back (green atoms are oxygen, blue are carbon, yellow are hydrogen). It acts like formaldehyde to a certain extent, creating familiar morphologic patterns of fixation. Immunoreactivity is fully maintained, just as in the formalin fixatives. Tissues become gently firmed but not over-hardened after fixation, making sectioning easier.

Stability of the reagent.

To overcome the acidification of the fixative the solution here adopted was linked to the addition of ethanol and glycol. The resulting 2% GAF in phosphate buffer 0.11 M pH 7.1-7,6 (hereafter GAF) is stable for at least 6 month at 4*C and at least 15 days at room temperature. An indicator (Phenol red) is added to give evidence to the slightly alkaline conditions of the reagent, by a pink color. Discard when a yellow color indicates acidic pH.

Safety

Data of the literature show that glyoxal holds a very low toxicity even though holding a similar reactivity to formaldehyde. Glyoxal is not volatile and all of the regulatory problems associated with formalin are nonexistent with glyoxal.

However, to be effective, no fixative can be totally safe and Glyoxal A.F. could fix your fingers and corneas if accidentally in contact, just as readily as it will your surgical specimens. Nitrile gloves and goggles are standard personal protective equipment (latex surgical gloves are ineffective against all chemical exposures).

The real issue of safety with formalin is carcinogenicity and inhalation exposure. Neither of these is a factor with Glyoxal which has such low vapour pressure that it cannot evaporate to any significant degree. Vapour monitoring is not needed, and all of the regulatory problems associated with formalin are non-existent with glyoxal.

Disposal

Disposal according to national law. After use, dispose as biological hazardous substance.

Glyoxal itself is not an EPA-listed hazardous waste. In the concentration present in these products, it does not possess any characteristics that qualify it as a hazardous waste by the EPA. It is the ethanol and glycol that qualify Glyoxal A.F. as hazardous waste. Glyoxal A.F. is not ignitable.