

	Operating mode	Réf : MO-PHIVLG_GUS_staining_01 Version 01 date : 06/07/2020 page 1/2
	GUS staining	

1. Object

GUS staining

2. Health and safety

Wear lab coat and gloves and work under the fume hood

3. Reagents (chemicals and biological)

	Formaldehyde (cas : 50-00-0)
	Formamide (68-12-2)
	X-Gluc(5-bromo-4-chloro-3-indolyl)-β-D-glucuronic acid, cas : 114162-64-0)
	Ethanol absolute

Phosphate Buffer NaPO₄ 100 mM pH7

For 250 ml

Na₂HPO₄ 1M : 14.4 ml

NaH₂PO₄ 1M: 10.6 ml

H₂O : 225 ml

4. Operating Mode

. Prefixation

Tissue were collected and then fixed with:

NaPO₄ 50 mM pH7

Formaldehyde 1.5 % (use formaldehyde 37 % as a stock solution)

TritonX100 0.05%

Make the solution ex tempore

Incubation for 30 to 45 min

Vacuum infiltration (5' to 30 ')

Wash sample with phosphate buffer 50 mM pH 7, repeat the step 3 X

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	Operating mode	Réf : MO-PHIVLG_GUS_staining_01
	GUS staining	Version 01 date : 06/07/2020 page 2/2

2. staining

Incubate the sample in:

	<u>For 10 ml</u>
NaPO ₄ 50 mM pH7	10 ml
Ferrocyanide 0.5 mM	100µl (stock solution ferro 50 mM 0.21g/10 ml)
Ferricyanide 0.5 mM	100µl (stock solution ferri 50 mM 0.16 g /10 ml)
TritonX100 0.05%	
XGluc 1mM	100 µl (X-Gluc 100 mM / 52.2 mg.ml ⁻¹ in dimethylformamide)
Make the solution ex temp	
Incubation in dark at 37°C (15 min to Overnight). Vacuum could be used for the first min.	

Wash sample with Phosphate buffer 50 mM pH 7.

3. Tissue Fixation (optional)

This step is necessary for embedding in resin to make thin sectioning

Fixation solution (80 ml) :

	<u>80 ml</u>
Phosphate buffer 0,2M pH7.2	40 ml
Formaldehyde 2 %	10 ml (formaldehyde vial 16%)
Glutaraldehyde 1 %	3,2 ml (stock solution 25 %)
Cafein (1% w/V)	0,8 g
H ₂ O	26,8ml

Aliquot and store at – 20°C

Incubate the samples in the fixation buffer

Optional : add 1 drop of tween 80

Put the tubes in a vacuum 30 min

Store the samples at 4°C, 48 h

Transfer the sample in Ethanol 50 %

Follow the dehydration protocol

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Name : Fonction : signature :	Carine Alcon		

	Operating mode	Réf : MO-PHIVLG_GUS_staining_01 Version 01 date : 06/07/2020 page 3/2
	GUS staining	

4. Dehydration

Ethanol 50 %	> 30 min	
Ethanol 70 %	> 30 min	
Ethanol 70 %	> 1 h	at this step you can store the sample at 4°C
Ethanol 95 %	> 30 min	
Ethanol 95 %	> 1 h	
Ethanol 100 %	> 30 min	
Ethanol 100 %	> 2 h	at this step you can store the sample at 4°C

Optional: for sample with a cuticule, and only for resin embedding step:

Ethanol 100% / Butanol 100 %	> 1h	
Butanol 100 %	> 2h	at this step you can store the sample at 4°C

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	Operating mode	Réf : MO-PHIVLG_GUS_staining_01 Version 01 date : 06/07/2020 page 4/2
	GUS staining	