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Product Information

β-Glucuronidase Reporter Gene Staining Kit

Catalog Number **GUSS**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Reporter genes are markers widely used for analysis of mutationally altered genes as well as gene regulation and localization. The expressed reporter genes are detected by biochemical activity assay, immunological assay, or by histochemical staining of tissue sections or cells.[1]

The *E. coli* GUS (β-glucuronidase) gene is extensively used as a gene fusion marker for analysis of gene expression in transformed plants. The GUS reporter gene system has many advantages including the stability of the expressed *E. coli* GUS enzyme and the low intrinsic activity of GUS in higher plants. The enzyme does not interfere with normal plant metabolism, and it remains active when fused to other proteins at its N-terminus making it useful for study of organelle transport in plants.[2-5] GUS transformed plants develop normally, and are healthy and fertile.

The *E. coli* β-glucuronidase enzyme hydrolyzes D-glucuronic acid conjugated through a β-O-glycosidic linkage to an aglycone. The compound 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-GlcA) is a GUS substrate, which upon hydrolysis produces an indigo-blue precipitate. It is used in histochemical staining of plant tissues expressing the *E. coli* GUS enzyme.

X-GlcA is applied to tissue sections in phosphate buffer, pH 7.0, containing potassium ferricyanide and potassium ferrocyanide. The substrate penetrates the tissue and is hydrolyzed by the β-glucuronidase. The indolyl moiety obtained is then oxidized to an indoxyl radical in a reaction catalyzed by the ferricyanide/ ferrocyanide mixture. The indoxyl moiety dimerizes to form an insoluble indigo-blue derivative.

Components

Sufficient reagents are provided to prepare 100 ml of staining solution.

Reagent A 200 mM sodium phosphate, pH 7.0 with 4 mM EDTA (Catalog Number R6147)	50 ml
Reagent B 100 mM potassium ferricyanide (Catalog Number R6272)	0.2 ml
Reagent C 100 mM potassium ferrocyanide (Catalog Number R6397)	0.2 ml
2× Fixation Buffer 20 mM MES, pH 5.6, with 600 mM mannitol and 0.6% formaldehyde (Catalog Number F2422)	25 ml
5-Bromo-4-chloro-3-indolyl β-D-glucuronide (X-GlcA), cyclohexylammonium salt (Catalog Number B6650)	80 mg

Reagents and Equipment Required but not Provided

- Dimethyl Sulfoxide (DMSO, Catalog Number D8779)
- Ethanol, spectrophotometric grade (Catalog Number 245119)
- Methanol (Catalog Number 494437)
- Desiccator
- Vacuum desiccator
- Incubator
- Containers to hold the tissue, such as 3–5 ml clear glass or plastic vials, or multiwell plates
- Petri dish
- Scalpel
- Forceps

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

Preparation Instructions
1. X-GlcA Solution - Dissolve X-GlcA in DMSO at a concentration of 350 mg/ml. A clear yellow solution is obtained with gentle vortexing. Use the proper amount of X-GlcA to prepare the desired volume of Staining Solution. For example, for 10 ml of Staining Solution, dissolve 7.0 mg of X-GlcA in 20 µl of DMSO.

2. Staining Solution - Add the components in the following order:

Component	Volume
Reagent A	2.5 ml
Reagent B	10 µl
Reagent C	10 µl
Deionized water	5.5 ml
Methanol	2.0 ml
X-GlcA Solution	20 µl
Total volume	~10 ml

   The Staining Solution may be stored at 2–8 °C in the dark for one month.

3. Fixation Solution (optional) - Dilute 2× Fixation Buffer 1:1 with deionized water.

4. Wash Solution (optional) - Dilute Reagent A 1:20 with deionized water.

Storage/Stability
The kit ships on dry ice and storage at –20 °C is recommended.

Procedure[3,4]
1. Transfer plant tissue to the 3–5 ml clear vials or multiwell plate. If optional fixation step is not necessary, proceed to step 5.
2. Add Fixation Solution. Make sure the tissue is covered with the solution.
3. Incubate at room temperature for 45 minutes.
4. Pour off the Fixation Solution and wash three times with Wash Solution, leaving the tissue in contact with the Wash Solution for one minute each time.
5. Add Staining Solution. Make sure that the tissue is covered with the solution.
6. Degas the vials or plate for two minutes in a vacuum desiccator. This step serves to remove air from the plant tissue and facilitate uptake of the staining solution.
7. Cover the containers with lids and incubate at 37 °C for up to 24 hours. A blue stain develops with time. When expression is high the solution becomes blue due to leakage of the blue reaction product from the tissue.
8. Remove the green chlorophyll by destaining the sample with ethanol. Leave the tissue in contact with the ethanol for 1–3 hours. More than one destaining step may be necessary.
9. Store the tissue in ethanol.

References

1. Kain, S.R., and Ganguly, S., Uses of Fusion Genes in Mammalian Transfection, in Current Protocols in Molecular Biology, vol. 1, suppl. 36, Ausubel, F.M. et al., eds., John Wiley & Sons, (New York, NY: 1996) p. 9.6.1.
2. Jefferson, R.A., Nature, **342**, 837 (1989).
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4. Kosugi, S. et al., Plant Science, **70**, 133 (1990).
5. Gallagher, S.R. et al., GUS Protocols: Using the GUS Gene as a Reporter of Gene Expression, Academic Press, (San Diego, CA: 1992).

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