V02

GUS染色试剂盒（即用型）

货号：G3061
规格：20mL/100mL
保存：-20℃，避光保存，有效期 6 个月。

产品组成：

名称	20mL	100mL	保存
试剂(A1): X-Gluc干粉	14mg	2×35mg	-20℃，避光
试剂(A2): X-Gluc溶剂	0.4mL	2×1mL	室温，避光
试剂(B): GUS缓冲液	20mL	2×50mL	2-8℃，避光

产品介绍：

X-Gluc（X-GlcA）分子量为 521.8，CAS 号为 18656-96-7，是检测大肠杆菌中 GUS 基因的底物，可快速检测植物中 GUS 基因融合标记。GUS 染色液在适宜的反应条件下，β-葡萄糖苷酶(GUS)可将 X-Gluc 水解成蓝色物质，该物质不溶解于转基因的细胞核组织中的靛蓝物质，具有 GUS 活性的部位或位点呈现蓝色或蓝色斑点，可用肉眼或显微镜观察到。GUS 染色液多用于转基因植物的 GUS 基因表达分析。

操作步骤：(*仅供参考*)

1、配制 X- Gluc 储备液(50×)：对于 20mL 规格，吸取 0.4mL X-Gluc 溶剂加至 14mg X-Gluc 干粉瓶中，使其完全溶解即为X-Gluc储备液(50×)，对于100mL规格，吸取1管1mL X-Gluc溶剂加至1瓶35mg X-Gluc干粉瓶中，使其完全溶解即为 X-Gluc 储备液(50×)，然后分装成小规格包装置于-20℃或-80℃保存。*（见注意事项 2）*

2、配制 X- Gluc 染色液：取适量 X-Gluc 储备液(50×)和 GUS Buffer，按 1:50 比例充分混匀，配制成 X- Gluc 染色液，如取 200ul X-Gluc 储备液(50×)加入到 10mL GUS 缓冲液中，即配成 10mL GUS 染色液，该染色液最好现配现用，短期可 4℃保存 3 天。

3、取适量待染叶片等组织加入适量 GUS 染色液，使 GUS 染色液完全浸没组织。

4、37℃孵育 1-24h。随着孵育时间的延长，蓝色渐渐出现，当表达量较高时，GUS 活性的部位或位点呈现蓝色或蓝色斑点。

5、用 70%乙醇脱去样本的叶绿素，一般样本浸没于乙醇 1-3h，至阴性对照呈白色。如有必要可重复该脱色步骤，以便彻底清除叶绿素。样本保存于乙醇中，可用肉眼或普通光学显微镜下观察，白色背景上的蓝色即为 GUS 表达位点。

注意事项：

1、配制好的 GUS 染色液可以 4℃避光保存 3 天。

2、X-Gluc 储备液(50×)应避免反复冻融，否则染色效率会下降。

3、由于组织特异性等原因，蓝色颜色反应可能不完全一致，应注意摸索具体实验条件。拟南芥的根、花和叶片以及烟草幼苗的根就可以不作任何预处理而直接染色。但是像烟草和马铃薯这些植物的茎和叶就必须在染色前切成薄片（1-3mm）。当操作大的组织和样品时，可以选用真空渗入法来帮助底物渗入细胞,建议使用 GUS 染色试剂盒（G3060）。

4、为了您的安全和健康，请穿实验服并戴一次性手套操作。

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GUS Stain Kit, Ready-To-Use

Cat: G3061
Size: 20mL/100mL
Storage:-20℃, avoid light, valid for 6 months.

Kit Components

Reagent	20mL	100mL	Storage
Reagent(A1): X-Gluc Powder	14mg	2×35mg	-20℃, avoid light
Reagent(A2): X-Gluc Solvent	0.4mL	2×1mL	RT, avoid light
Reagent(B): GUS Buffer	20mL	2×50mL	2-8℃, avoid light

Introduction

X-Gluc(X-GlcA) has a molecular weight of 521.8 and CAS number of 18656-96-7. It is a substrate for the detection of GUS gene in Bacillus coli and can rapidly detect GUS gene fusion markers in plants . Under suitable reaction conditions, β-glucosidase (GUS) can hydrolyze X-Gluc into blue substance, which is not dissolved in Indigo substance in transgenic nuclear tissue. The sites or sites with GUS activity show blue or blue spots, which can be observed by naked eye or microscope. The GUS Staining Kit is mainly used for GUS gene expression analysis of transgenic plants.

Protocol(*for reference only*)

1. Prepare X-Gluc Solution(50×):For the 20mL specification, take 0.4mL of X-Gluc Solvent and add it into a 14mg X-Gluc Powder bottle to dissolve completely, which is the X-Gluc Solution(50×). For the 100mL specification, take 1 tube of 1mL X-Gluc Solvent and add it into a 35mg X-Gluc Powder bottle to dissolve completely, which is the X-Gluc Solution(50×). Then divide it into small size packages and store them at -20 ℃ or -80 ℃. *(See Note 2)*
2. Prepare GUS Solution: take appropriate X-Gluc Solution(50×) and GUS Buffer , mix fully as the radio of 1:50 to form GUS Solution. Such as mixing 200ul X-Gluc Solution(50×) and 10mL GUS Buffer to form 10mL GUS Solution. The solution is best to prepare before use and can store for 3 days at 4℃.
3. Take appropriate leaves and other tissues to be dyed and add appropriate GUS Solution to completely submerge the tissue.
4. Incubate at 37 ℃ for1-24h. With the increase of incubation time, blue gradually appears. When the expression level is high, the sites or sites of GUS activity show blue or blue spots.
5. Remove the chlorophyll of the sample with 75% ethanol, and generally soak the sample in ethanol for 1-3h until the negative control shows white color. If necessary, repeat the decolorization step to completely remove chlorophyll. The samples are stored in ethanol and can be observed by naked eye or ordinary optical microscope. Blue on white background is GUS expression site.

Note

1. The prepared GUS Solution can be stored for half a month at 4 ℃ in dark.
2. X-Gluc Solution(50×) should avoid repeated freezing and thawing, otherwise the dyeing efficiency will decrease.
3. Due to the tissue specificity and other reasons, the blue color reaction may not be completely consistent, so pay attention to explore the specific experimental conditions. The roots, flowers and leaves of arabidopsis and the roots of tobacco seedlings can be dyed directly without any pretreatment. But the stems and leaves of plants like tobacco and potato must be cut into slices (1-3mm) before dyeing. When handling with large tissues and samples, vacuum infiltration method can be used to help substrate penetrate into cells. GUS Stain Kit (G3060) is recommended.
4. For your safety and health, please wear lab coats and disposable gloves when operating.