GUS染色试剂盒

货号： G3060
规格： 100mL
保存： -20℃, 避光保存, 有效期 6 个月。

产品组成：

名称	规格	保存
试剂(A): GUS缓冲液A	50mL	室温
试剂(B): GUS缓冲液B	0.2mL	2-8℃，避光
试剂(C): GUS缓冲液C	0.2mL	2-8℃，避光
试剂(D): 2×缓冲固定液	25mL	室温，避光
试剂(E): X-GlcA	80mg	-20℃，避光
试剂(F): X-GlcA 溶剂	1mL	室温，避光
试剂(G): 去离子水	100mL	室温

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- GlcA 工作液：取 X-GlcA 溶剂 229ul 加入至 80mg X-GlcA 中或取适量上述 2 种物质溶解，使其浓度达到 350mg/mL，轻轻混匀，即为 X- GlcA 工作液，分装后，-20℃避光保存。
2. 按下列比例配制 GUS 染色液：

组分	体积
GUS缓冲液A	2.5mL
GUS缓冲液B	10ul
GUS缓冲液C	10ul
去离子水	5.5mL
甲醇	2.0mL
X-GlcA工作液	20ul
总体积	约10mL

3. 固定(固定不是必须的步骤，如果不需要固定，直接进行操作步骤 4)：
   ① 取转基因植物组织，加入到 3-5mL 左右清洁小瓶或多孔板中。
   ② 取适量 2×缓冲固定液与去离子水等量稀释即获得 1×缓冲固定液。加入 3-5 倍组织体积的 1×缓冲固定液没过组织，室温孵育 45min，弃液。
   ③ 配制洗涤液：按 GUS 缓冲液 A:去离子水=1:20 稀释，即为洗涤液。用配制好的洗涤液漂洗 3 次。每次 1min。
4. 加入适量 GUS 染色液，使 GUS 染色液完全浸没组织。
5. 用真空泵抽取小瓶或多孔板中的气体，抽取时间应大于 2min。该步骤是为了抽取植物组织内的气体，并使染色液更容易进入组织内。
6. 立即盖紧瓶子或多孔板，37℃孵育 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。
7. 用 75%乙醇脱去样本的叶绿素，一般样本浸没于乙醇 1-3h。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。

注意事项：

1. 配制好的 GUS 染色液可以 2-8℃避光保存半个月。
2. X-GlcA 工作液应避免反复冻融，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。
4. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

GUS Stain Kit

Cat: G3060
Size:100mL
Storage: -20℃, avoid light, valid for 6 months.

Kit Components

Reagent	Size	Storage
Reagent(A): GUS Buffer A	50mL	RT
Reagent(B): GUS Buffer B	0.2mL	2-8℃, avoid light
Reagent(C): GUS Buffer C	0.2mL	2-8℃, avoid light
Reagent(D): 2×Fixation Buffer	25mL	RT, avoid light
Reagent(E): X-GlcA	80mg	-20℃, avoid light
Reagent(F): X-GlcA Solvent	1mL	RT, avoid light
Reagent(G):Deionized Water	100mL	RT

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- GlcA Solution: add 229ul of X-GlcA Solvent to 80mg X-GlcA, or take appropriate amount of the above two substances to dissolve, so that the concentration reaches 350mg/mL, mix them gently to form X-GlcA Solution. After distributing, store them in dark at - 20 ℃.
2. Prepare GUS Solution according to the radio as follows.

Reagent	**volume**
GUS Buffer A	2.5mL
GUS Buffer B	10ul
GUS Buffer C	10ul
Deionized water	5.5mL
Methanol	2.0mL
X-GlcA Solution	20ul
Total volume	Approximately 10mL

3. Fixation(Fixation is not a necessary step. If fixation is not required, proceed to step 4):
   ①Take the transgenic plant tissue and add it into a about 3-5mL of clean vial or porous plate.
   ②Take equal amount of 2×Fixation Buffer and Deionized Water to mix to form 1×Fixation Buffer. Add 1×Fixation Buffer to completely submerge the tissue. Incubate at room temperature for 45min, discard the liquid.
   ③Prepare Washing Solution: mix GUS Buffer A with Deionized Water according to the radio of 1:20 to form Washing Solution. Rinse 3 times with the prepared Washing Solution for 1 min each time.
4. Add appropriate GUS Solution to completely submerge the tissue.
5. Use a vacuum pump to extract the gas in a vial or porous plate, and the extraction time shall be more than 2min. The purpose of this step is to extract gas from plant tissues and make the dye more easily enter the tissues.
6. Immediately cover the vial or porous plate and incubate at 37 ℃ for 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.

7. Remove the chlorophyll of the sample with 75% ethanol, and generally soak the sample in ethanol for 1-3h. 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.

Note

1. The prepared GUS Solution can be stored for half a month at 2-8 ℃ in dark.
2. X-GlcA Solution 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.
4. For your safety and health, please wear experimental clothes and disposable gloves.