V02

植物木栓化细胞壁染色液（苏丹法）

货号：G1525
规格：50mL
保存：室温，避光保存，有效期 1 年。

产品介绍：

植物细胞壁作为植物组织的防御层，由多糖、蛋白质、木质素，脂类化合物以及矿物质等物质组成，为组织提供机械支持，在物质运输、细胞识别和细胞生长等过程中起重要作用。细胞壁木栓化形成是由于增加了脂肪性化合物木栓质，细胞壁不易透气和逐水，最后造成细胞内原生质体的消失，由脂肪化合物进行填充。

植物木栓化细胞壁染色液（苏丹法）可用于徒手切片、冰冻切片与经植物组织固定液处理过的石蜡切片样本的角质化细胞壁或者栓质化细胞壁的染色观察，角质或栓质化细胞壁被染成橘红色。

操作步骤：(*仅供参考*)

对于冰冻切片

1. **取材：**取新鲜植物组织根、茎、叶进行横切，对于较大的叶片，大小以不超过 1cm × 1cm 为宜。
2. **固定：**将组织浸于 OCT 包埋剂中，然后带着包埋剂进行负压处理约 20min，至组织被完全浸透。液氮速冻后置于-80℃保存。
3. **制片：**冰冻切片厚度以 10um 为宜，稍晾干后用预冷的植物组织固定液或丙酮稍固定后置于-20℃保存。
4. **染色：**水洗复温后先用 70%乙醇处理切片 1-2min，植物木栓化细胞壁染色液（苏丹法）浸染 5-10min 后，70%乙醇快速冲洗分化 2-3s，再用蒸馏水冲洗直至无染料脱出。（见注意事项 4）
5. **镜检：**切片带水用甘油明胶封片，镜下观察。

对于石蜡切片

1. **取材：**取新鲜植物组织根、茎、叶进行横切，对于较大的叶片，大小以不超过 1cm × 1cm 为宜。
2. **固定：**将组织浸于 AAF 固定液固定 4h 以上，然后带着固定液进行负压处理约 20min，至组织下沉不再排出气体。转于 70%乙醇中平衡 1h 充分洗脱固定液，然后进行正常的脱水、透明和包埋处理。（见注意事项 2）
3. **制片：**石蜡切片厚度以 3-5um 为宜，正常脱蜡至水。
4. **染色：**先用 70%乙醇处理切片 1-2min，植物木栓化细胞壁染色液（苏丹法）浸染 5-10min 后，70%乙醇快速冲洗分化 2-3s，再用蒸馏水冲洗直至无染料脱出。（见注意事项 4）
5. **镜检：**切片带水用甘油明胶封片，镜下观察。

染色结果：

栓质化细胞壁	橘红或红色
背景	无色或淡黄色

注意事项：

1. 对于较小组织样本（直径小于 1.5mm 的茎或厚度小于 1mm 的叶片）亦可不进行负压处理。
2. 若组织不能及时负压处理，可浸于 AAF 固定液固定过夜再进行负压处理，流程同步骤 2。
3. 若想加速染色，可以先将染液在 60℃中预热，升温可以促进染液与细胞壁中木栓物质的结合。
4. 本试剂易挥发，最好采用浸染方式，防止染液沉积在组织和切片上不易洗掉。
5. 此染色液可以重复使用 4-5 次，不用时及时扣上盖子防止试剂挥发。
6. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

Tel: 400-968-6088 https://www.solarbio.com E-mail: sales-china@solarbio.com
Address: No. 85, Liandong U Valley, Middle Zone, 101102, Tongzhou Dist, Beijing, China

Plant Cork Cell Wall Stain Solution (Sudan Method)

Cat: G1525
Size: 50mL
Storage: RT, avoid light, valid for 1 year.

Introduction

As the defense layer of plant tissue, plant cell wall is composed of polysaccharides, proteins, lignin, lipid compounds and minerals. It provides mechanical support for tissue and plays an important role in material transportation, cell recognition and cell growth. The formation of cell wall corking is due to the increase of fatty compound cork, which makes the cell wall difficult to breathe and drain water. Finally, the Protoplast in the cell disappears and is filled by fatty compounds.

Plant Cork Cell Wall Stain Solution (Sudan Method)can be used to observe the staining of keratinized cell wall or suberized cell wall of bare handed sections, frozen sections and paraffin sections treated with plant tissue fixing solution. Keratinized cell wall or suberized cell wall can be dyed orange color.

Protocol(*for reference only*)

For frozen sections

1. **Sampling:** take the roots, stems and leaves of fresh plant tissues for cross cutting. For larger leaves, the size shall not exceed 1cm × 1cm is appropriate.
2. **Fixation:** immerse the tissue in OCT embedding agent, and then carry the embedding agent for negative pressure treatment for about 20min until the tissue is completely soaked. After quick freezing with liquid nitrogen, store at - 80 ℃.
3. **Section:** the thickness of frozen section should be 10um. After drying, slightly fix tissues with precooled plant tissue fixing solution or acetone and stored at - 20 ℃.
4. **Dyeing:** after rewarming, firstly treat the slices with 70% ethanol for 1-2min, soak in Plant Cork Cell Wall Stain Solution (Sudan Method) for 5-10min, quickly rinse the differentiation with 70% ethanol for 2-3s, and then rinse with distilled water until there is no dye. (see note 4)
5. **Microscopic examination:** slice with water, seal with glycerin gelatin and observe under microscope.

For paraffin sections

1. **Sampling:** take the roots, stems and leaves of fresh plant tissues for cross cutting. For larger leaves, the size shall not exceed 1cm × 1cm is appropriate.
2. **Fixation:** immerse the tissue in AAF fixative for more than 4h, and then conduct negative pressure treatment with the fixative for about 20min until the tissue sinks and no gas is discharged. Transfer to 70% ethanol for equilibrium for 1h, fully elute the stationary solution, and then carry out normal dehydration, transparency and embedding treatment. (see Note 2)
3. **Production:** the thickness of paraffin section should be 3-5um, and it should be dewaxed to water normally.
4. **Dyeing:** firstly treat the slices with 70% ethanol for 1-2min, soak in Plant Cork Cell Wall Stain Solution(Sudan Method) for 5-10min, quickly wash with 70% ethanol for 2-3s, and then rinse with distilled water until there is no dye. (see note 4)
5. **Microscopic examination:** slice with water, seal with glycerin gelatin and observe under microscope.

Result

Suberized Cell Wall	Orange Red
Background	Colorless or Light Yellow

Note

1. For small tissue samples (stems with diameter less than 1.5mm or leaves with thickness less than 1mm), negative pressure treatment can also be avoided.
2. If the tissue cannot be treated with negative pressure in time, can immerse tissue in AAF fixative for fixation overnight and then treat with negative pressure. Conduct the process with step 2.
3. If you want to speed up the dyeing, you can first preheat the dye at 60 ℃, and the heating can promote the combination of the dye and the cork in the cell wall.
4. The reagent is volatile, and it is best to use immersion dyeing to prevent the dye from depositing on the tissue and section, which is not easy to wash off.
5. The dye can be reused for 4-5 times. When not in use, close the cover in time to prevent reagent volatilization.
6. For your safety and health, please wear experimental clothes and disposable gloves.