V02

β-半乳糖苷酶染色试剂盒

货号：G1580
规格：100T
保存：-20℃，避光保存，有效期 1 年。

产品组成：

名称	100T	保存
试剂(A): β-Gal 固定液	100mL	2-8℃,避光
试剂(B): X-Gal 溶液	5mL	-20℃,避光
试剂(C): β-Gal 染色液 A	1mL	2-8℃,避光
试剂(D): β-Gal 染色液 B	1mL	2-8℃,避光
试剂(E): β-Gal 染色液 C	100mL	2-8℃
按照试剂 B:C:D:E=5:1:1:93 的比例配置染色工作液，现用现配。		

产品介绍：

β-半乳糖苷酶染色试剂盒是一种基于衰老时衰老相关半乳糖苷酶（SA-β-Gal）活性水平上调而对衰老细胞或组织进行染色检测的试剂盒。在普通的光学显微镜下就可以观测到细胞或组织的衰老情况。细胞衰老也被认为是生物体抑制肿瘤的一种方式，同时也是生物体老化的一种潜在原因。本试剂盒可以用于培养细胞的衰老检测，也可以用于组织切片的衰老检测。

β-半乳糖苷酶染色试剂盒以 X-Gal 为底物，在衰老特异性的β-半乳糖苷酶催化下会生成深蓝色产物，光学显微镜下很容易观察到变成蓝色的表达β-半乳糖苷酶的细胞或组织。本试剂盒仅染色衰老细胞，对正常培养的衰老前的细胞、静止期细胞、永生细胞或肿瘤细胞等不会染色。对于组织切片或组织块，可以检测的样品数量视样品的大小而定，对于普通的切片也至少足够检测 100 个样品，使用 6 孔板测定，足够测定 100 个样品。

操作步骤：(*仅供参考*)

注意：配置染色工作液推荐使用聚丙烯(PP)容器，不能使用聚苯乙烯(PS)容器配制染色工作液。
染色过程中的 37℃孵育不能在二氧化碳培养箱中进行，易造成假阳性染色。

(一)贴壁细胞染色：

1. 对于 6 孔板中培养的细胞，吸除细胞培养液，用 PBS 洗涤 1 次，加入 1ml β-Gal 固定液，室温固定 15min。对于其它类型的培养板，固定液及后续溶液的用量参照此比例进行操作。
2. 吸除细胞固定液，用 1×PBS 洗涤细胞 3 次，每次 3min。（优选步骤见注意事项 3）
3. 按照比例配置染色工作液。吸除β-Gal 清洗液，每孔加入 1ml 染色工作液。
4. 37℃孵育过夜 12-24h，可以用封板膜或保鲜膜封住 6 孔板防止蒸发。
5. 普通光学显微镜下观察。如不能及时观察计数，可以去除染色工作液，加入 2ml PBS，2-8℃可以保存数天；或者加上水性明胶封片剂封片后，可以保存较长时间。

(二)悬浮细胞染色：

1. 离心收集细胞至 1.5ml 离心管内，用 PBS 洗涤 1 次，加入 1ml β-Gal 固定液，室温固定 15min。固定时可以在摇床上缓慢摇动，以避免细胞结成团块。
2. 离心，吸除细胞固定液，用 1×PBS 洗涤细胞 3 次，每次 3min。（优选步骤见注意事项 3）
3. 按照比例配置染色工作液。离心，吸除β-Gal 清洗液，每管加入 0.5-1ml 染色工作液。37℃孵育过夜 12-24h。
4. 取部分染色后的细胞，滴加到载玻片上或 6 孔板内，普通光学显微镜下观察。如不能及时观察计数，可以离心，去除染色工作液，然后加入 1ml PBS，2-8℃可以保存数天。如果离心，取细胞用于涂片，加上封片液封片后，2-8℃可以保存较长时间。

(三)组织切片染色：

1. 对于酶保护处理过的冷冻切片，直接按照以下步骤进行。（见注意事项 4）
2. 滴加适当体积的β-Gal 固定液，以充分盖住组织为宜，室温固定不少于 15min。

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

3. 用 PBS 浸泡洗涤组织 3 次，每次不少于 5min。（优选步骤见注意事项 3）
4. 按照比例配置染色工作液。滴加适当量的染色工作液。
5. 37℃孵育过夜 12-24h。建议使用湿盒防止挥发或把整个切片浸泡在染色工作液中。
6. 普通光学显微镜下观察。如不能及时观察，可使用水性明胶封片剂封片保存 。

注意事项：

1. β-Gal 固定液有一定的腐蚀性和毒性，操作时请注意防护。
2. β-半乳糖苷酶染色反应依赖于特定的 pH 条件，不能在二氧化碳培养箱中进行染色反应。用于细胞培养的二氧化碳培养箱中较高浓度的二氧化碳会影响染色工作液的 pH 值，而导致染色失败。
3. 优选步骤，在滴加染色工作液之前使用试剂(E): β-Gal 染色液 C 替代 1×PBS 进行清洗和孵育。
4. 半乳糖苷酶稳定性较差，在使用多聚甲醛固定或制备石蜡切片时极易灭活，建议制备速冻切片，或使用酶保护型专用固定液：G2195-半乳糖苷酶定色剂进行固定和冰冻切片制备。组织浸于半乳糖苷酶定色剂中可稳定酶活至少一周。
5. 配制染色工作液时需使用聚丙烯(PP)容器或玻璃容器，不宜使用聚苯乙烯(PS)容器。但染色时对容器材质无要求，例如普通的 6 孔板就可以用作染色的容器。
6. 爬片或涂片如需长期保存可染色后蒸馏水洗 2 次，倾去多余液体晾干后中性树胶封片。
7. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

V02

Senescence-Associated β-Galactosidase(SA-β-Gal)Stain Kit

Cat: G1580
Size: 100T
Storage:-20℃,avoid light, valid for 1 year.

Kit Components

Reagent	100T	Storage
Reagent(A): β-Gal Fixative	100mL	2-8℃,avoid light
Reagent(B): X-Gal Solution	5mL	-20℃,avoid light
Reagent(C): β-Gal Stain Solution A	1mL	2-8℃,avoid light
Reagent(D): β-Gal Stain Solution B	1mL	2-8℃,avoid light
Reagent(E): β-Gal Stain Solution C	100mL	2-8℃
Mix Reagent B,C,D,E, in proportion of 5:1:1:93 as Dyeing Working Solution. It is ready to use.		

Introduction

The Senescence β-Galactosidase Staining Kit is a staining kit for senile cells or tissues based on the up-regulation of SA-β-Gal(senescence-associated β-galactosidase) activity during aging. Aging of cells or tissues can be observed under optical microscopes. Cell aging is also considered as a way for organisms to inhibit tumors and a potential cause of organism aging. The kit can be used for senescence detection of cultured cells and tissue sections.

Using X-Gal as substrate, the Senescence β-Galactosidase Staining Kit produces dark blue products catalyzed by aging-specific β-galactosidase. Cells or tissues expressing β-galactosidase that turn blue are easily observed under optical microscopy. This kit only stains senescent cells. For tissue sections or blocks, the number of samples that can be detected depends on the size of the sample. For ordinary sections or 6-well plate, at least 100 samples can be detected.

Protocol(*for reference only*)

Note: It is recommended to use polypropylene (PP) container to prepare dyeing working solution, but polystyrene (PS) container cannot be used to prepare dyeing working solution.

The incubation at 37 ℃ during dyeing could not be carried out in carbon dioxide incubator.

Adherent Cells Stain

1. For cells cultured in 6-well plates,remove the cell culture medium and wash once with PBS or HBSS. Add 1 ml β-Gal Fixative to each pore and fix at room temperature for 15 min. For other types of culture plates, the amount of fixative solution and subsequent solution is operated according to this ratio.
2. Remove β-Gal Fixative and wash cells 3 times with 1×PBS for 3 min each time. (*priority step see note 3*)
3. Remove washing solution and add 1 ml Dyeing Working Solution to each hole.
4. When incubate overnight for 12-24h at 37℃, the 6-well plate can be sealed with parafilm to prevent evaporation.
5. View under the optical microscope. If you can't view and count in time, you can remove the Dyeing Working Solution, add 2ml PBS, and store it at 2-8 ℃ for several days; moreover, it can be stored for a long time after sealing with aqueous gelatin sealing agent.

Suspension Cell Stain

1. Collect the cells into 1.5ml eppendorf tube by centrifugation, wash once with PBS or HBSS, and fix at room temperature for 15 min with 1 ml β-Gal Fixative. Slowly shake on the shaking table to avoid forming cell clumps.
2. Centrifuge and remove β-Gal Fixative, wash cells with 1×PBS 3 times for 3 min each time. (*priority step see note 3*)
3. Centrifuge and remove the washing solution then add 0.5-1 ml Dyeing Working Solution to each tube. Incubate overnight for 12-24h at 37℃.
4. Drip some stained cells onto section and view under optical microscope. If not view in time, centrifuge and remove the dyeing working solution then adding 1 ml PBS at 2-8℃ can keep for several days. If centrifuged, cells can also be taken for smears, and sealed with water based sealing agent, it can be stored for a long time at 2-8℃.

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

Tissue Sections Stain

1. For enzymatically protected frozen slices, follow the steps below. (See Note 4)
2. Add a suitable volume of β-Gal Fixative to cover the tissue adequately and fix it for more than 15 min. (*priority step see note 3*)
3. Wash the tissues 3 times with PBS for more than 5 min each.
4. Remove washing solution.
5. When incubate overnight for 12-24h at 37℃, if drip dyeing, it can be placed in the wet-box to prevent evaporation; if soak dyeing, soak the section in dye container for closed condition.
6. View under optical microscope. If not view in time, can seal with glycerol gelatin for storage.

Note

1. The β-Gal Fixative has certain corrosiveness and toxicity. Pay attention to the protection during operation.
2. The β- galactosidase staining reaction depends on specific pH conditions and cannot be performed in a carbon dioxide incubator. The high concentration of carbon dioxide in the carbon dioxide incubator used for cell culture can affect the pH value of the staining solution, leading to staining failure.
3. The priority step is using reagent E:β-Gal Stain Solution C to wash and incubate instead of 1×PBS before staining.
4. The stability of galactosidase is poor, and it is easy to inactivate when using paraformaldehyde to fix or prepare paraffin sections. It is recommended to prepare quick-frozen sections, or use enzyme protection special fixing solution: G2195-β-Galactosidase Colorimetric Solution for fixing and freezing sections. Tissue immersion in galactosidase fixative stabilizes enzyme activity for at least one week.
5. PP containers or glass containers should be used in the preparation of dyeing working solution instead of PS containers. But dyeing can be carried out in PS containers, such as 6-well plate.
6. If the climbing film or smear needs to be stored for a long time, it can be washed twice with distilled water after dyeing, and the neutral gum seal can be made after the excess liquid is poured out and dried.
7. For your safety and health, please wear experimental clothes and disposable gloves.