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细胞衰老β-半乳糖苷酶染色试剂盒

产品编号	产品名称	包装
C0602	细胞衰老β-半乳糖苷酶染色试剂盒	>100次

产品简介：

- 细胞衰老β-半乳糖苷酶染色试剂盒(Senescence β-Galactosidase Staining Kit)是一种基于衰老时衰老相关β-半乳糖苷酶(senescence-associated β-galactosidase; 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。本试剂盒可以用于培养细胞的衰老检测，也可以用于冰冻切片的衰老检测。
- 绝大多数正常细胞被认为仅有有限的分裂能力，在不能分裂后就进入衰老(senescence)状态。此时细胞仍然是存活的，但细胞的基因和蛋白的表达谱发生了很大改变。衰老的细胞不能在一些常规的刺激下再诱导细胞分裂，并且衰老细胞的细胞周期分布也比较特殊，不同于一些损伤诱导的细胞休眠，也不同于细胞生长接触抑制的情况。衰老细胞通常体积变大，并表达在pH6.0时有高酶活性的β-半乳糖苷酶(β-galactosidase)。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(aging)的一种潜在原因。
- 碧云天生产的细胞衰老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。从而在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。本产品染色的染色效果请参考图1。

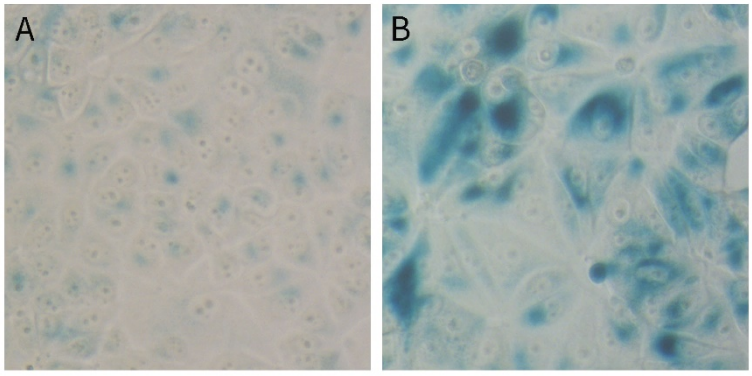
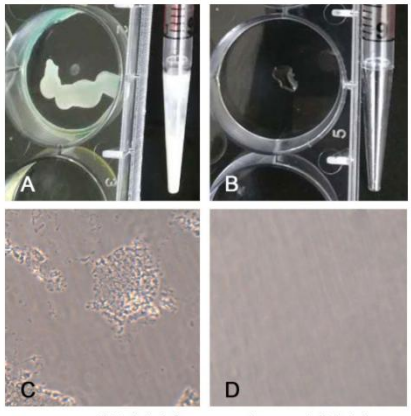


图1. 细胞衰老β-半乳糖苷酶染色试剂盒用于MCF-7细胞的染色效果图。图A是正常的MCF-7细胞，图B是MCF-7细胞用Etoposide (SC0173)处理诱导的衰老组。实际染色效果会因样品及实验条件的不同而存在差异，本图仅供参考。

- 本试剂盒仅染色衰老细胞，不会染色衰老前的细胞(presenescent cells)、静止期细胞(quiescent cells)、永生细胞(immortal cells)或肿瘤细胞。
- **主要特点：**本试剂盒经过多方面的优化，是同类产品中首创的能兼容普通的细胞培养用多孔板、移液管等聚苯乙烯类材质耗材或容器的试剂盒。本试剂盒可以有效避免由于和多孔板、移液管等的不兼容导致的染色偏弱、染色效果不稳定等情况。通常同类试剂盒要求使用可高温高压灭菌的聚丙烯(polypropylene)材质的耗材、容器或玻璃容器进行溶液的配制，而不能使用普通的多孔板、移液管等聚苯乙烯(polystyrene)类材质的容器或耗材，否则可能会出现絮状沉淀，影响实验观察。即使严格按照要求操作，也会在染色时间比较长的情况下，容易出现絮状物沉淀(参考图2)。本试剂盒经过多方面的优化，对耗材或容器的材质无特殊要求，可以兼容普通的多孔板和移液管等常用耗材和容器。而且配制的工作液不会产生沉淀或不溶物，使用更加便捷。



国际同类试剂盒　　碧云天试剂盒

图2. 本试剂盒优化前后的对比图。优化前，X-Gal溶液直接接触聚苯乙烯类材质的材料如移液管、多孔板等会产生明显的腐蚀(A图)，使用聚苯乙烯容器配制染色工作液后，在显微镜下观察有异常的絮状不溶物(C图)；优化后，X-Gal溶液直接接触聚苯乙烯类材质的材料观察不到有任何异常情况(B图)，使用聚苯乙烯容器配制染色工作液后，在显微镜下观察也没有任何异常情况(D图)。

- 如果使用6孔板检测，足够测定100个样品；使用24孔板测定，足够测定400个样品；使用96孔板测定，足够测定1000个样品。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。对于普通切片的滴染足够检测100个样品。

包装清单：

产品编号	产品名称	包装
C0602-1	β-半乳糖苷酶染色固定液	100ml
C0602-2	X-Gal溶液	5ml
C0602-3	β-半乳糖苷酶染色液A	1ml
C0602-4	β-半乳糖苷酶染色液B	1ml
C0602-5	β-半乳糖苷酶染色液C	100ml
—	说明书	1份

保存条件：

-20°C保存，一年有效。其中X-Gal溶液需避光保存。

注意事项：

- 加入染色工作液后，由于溶液蒸发或其它未知原因等因素，可能会有结晶形成而影响观察和拍摄照片，此时建议吸除染色工作液，加入适量70%乙醇进行洗涤，70%乙醇可在短时间内溶解结晶，待结晶溶解消失后再更换成PBS或生理盐水。70%乙醇的洗涤对染色效果没有任何影响。
- 在石蜡包埋的过程中，温度、固定液等因素可能会导致β-半乳糖苷酶失活，从而造成染色失败，因此本试剂盒不建议用于石蜡切片的衰老检测。如果一定要用于石蜡切片的检测，建议自行对实验条件进行一定的优化。
- β-半乳糖苷酶染色固定液对人体有毒、有腐蚀性，操作时请特别小心，并注意有效防护以避免直接接触人体或吸入体内。
- X-Gal溶液在-20°C或4°C保存会冻结，室温或37°C水浴2-5分钟并适当摇动即可完全融解。
- 细胞衰老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。
- 试剂解冻后或使用前如果有沉淀，必须在使用前确保沉淀全部溶解。β-半乳糖苷酶染色液B刚从试剂盒中取出时，管底可能存在少量沉淀，属正常现象，充分混匀或Vortex后，沉淀会全部溶解，并须确保在全部溶解后使用。配制染色工作液时，也可能有少量絮状沉淀出现，震荡混匀后就会完全溶解，且须确保全部溶解后才能使用。
- 使用96孔板等多孔板进行检测时，如果孵育过夜容易产生所谓的‘‘边缘效应’’(edge effect)，即多孔板四周的孔由于和外界最直接接触，易受外界环境影响，其中最明显的是四周细胞培养孔的蒸发效应。边缘效应会导致细胞生长不均匀、细胞分布不均一、培养液体积不一致、培养液中相关成分的浓度、pH值不一致。建议采取以下方法避免96孔板等多孔板的边缘效应：避免孵育过长时间，以避免蒸发等带来的边缘效应；弃用边缘孔并在弃用的边缘孔中加入等量的水、PBS或其他适当溶液；在多孔板非孔的凹陷处加入适量的水或其他适当溶液；将整块板放在湿盒中；使用防挥发盖；在实验设计时，实验样品最好进行随机分配，不要将某一组样品固定放在某个位置而引入可能的系统性误差。
- 需自备PBS或HBSS(Hanks Balanced Salt Solution)。
- 本产品仅限于专业人员的科学研究用，不得用于临床诊断或治疗，不得用于食品或药品，不得存放于普通住宅内。
- 为了您的安全和健康，请穿实验服并戴一次性手套操作。

使用说明：

1. 对于贴壁细胞：

a. 对于6孔板中培养的细胞，吸除细胞培养液，用PBS或HBSS洗涤1次，加入1毫升β-半乳糖苷酶染色固定液，室温固定15分钟。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。

b. 吸除细胞固定液，用PBS或HBSS洗涤细胞3次，每次3分钟。

c. 吸除PBS或HBSS，每孔加入1毫升染色工作液。染色工作液的配制方法参考表1。

表1. 染色工作液的配制方法。

β-半乳糖苷酶染色液A	10μl
β-半乳糖苷酶染色液B	10μl
β-半乳糖苷酶染色液C	930μl
X-Gal溶液	50μl

d. 37°C孵育过夜，可以用parafilm或保鲜膜封住6孔板防止蒸发。注意：37°C孵育不能在二氧化碳培养箱中进行。

e. 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2毫升PBS，4°C可以保存数天；或者加上封片液封片后，4°C可以保存较长时间。注：如果有结晶形成，请参考注意事项中的建议使用70%乙醇进行洗涤处理。

2. 对于悬浮细胞：

a. 离心收集细胞至1.5ml离心管内，用PBS或HBSS洗涤1次，加入1毫升β-半乳糖苷酶染色固定液，室温固定15分钟。固定时可

以在摇床上缓慢摇动，以避免细胞结成团块。

b. 离心，吸除细胞固定液，用PBS或HBSS洗涤细胞3次，每次3分钟。

c. 离心，吸除PBS或HBSS，每管加入0.5-1毫升染色工作液。染色工作液的配制方法参考表1。

d. 37°C孵育过夜。注意：37°C孵育不能在二氧化碳培养箱中进行。

e. 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1毫升PBS，4°C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，4°C可以保存较长时间。注：如果有结晶形成，请参考注意事项中的建议使用70%乙醇进行洗涤处理。

3. 对于冰冻切片：

a. 冰冻切片先进行复温，用PBS浸泡洗涤组织3次，每次不少于5分钟。

b. 加入适当体积的β-半乳糖苷酶染色固定液，以充分盖住组织为宜，室温固定不少于15分钟。

c. 用PBS浸泡洗涤组织3次，每次不少于5分钟。

d. 吸除PBS，加入适当量的染色工作液。染色工作液的配制方法参考表1。

e. 37°C孵育过夜，可以用parafilm或保鲜膜封住防止蒸发。最好把整个切片浸泡在染色工作液中。注意：37°C孵育不能在二氧化碳培养箱中进行。

f. 普通光学显微镜下观察。如不能及时观察，加上封片液封片后4°C可以保存较长时间。注：如果有结晶形成，请参考注意事项中的建议使用70%乙醇进行洗涤处理。

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