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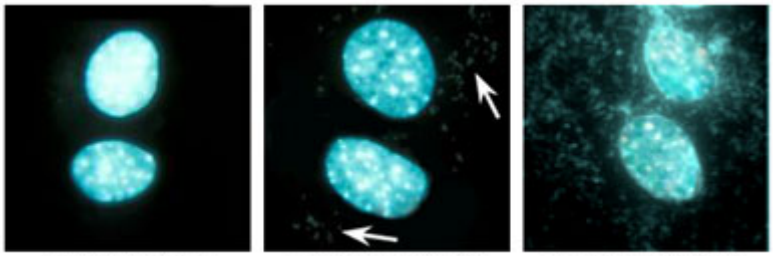
支原体染色检测试剂盒

产品编号	产品名称	包装
C0296	支原体染色检测试剂盒	>100次

产品简介:

- 支原体染色检测试剂盒(Mycoplasma Stain Assay Kit)是用于在培养细胞中原位染色检测支原体或其它原核生物的试剂盒。主要用于检测培养细胞中是否存在支原体污染。
- 培养细胞中的细菌污染、酵母污染或霉菌污染都在光学显微镜下可见，但支原体污染在光学显微镜下不可见，必须通过特定的检测方法进行检测。
- 检测支原体污染的方法有很多种，包括支原体分离培养、支原体特异酶检测、RT-PCR检测以及DNA荧光染色检测。上述检测方法中，除DNA荧光染色检测外操作步骤相对比较烦琐并且所需时间较长。本支原体染色检测试剂盒是通过Hoechst染色来检测支原体的。本试剂盒的荧光染色可快速、有效、高灵敏度地检测支原体污染。



- 本试剂盒的检测效果图参考上图。左图为无支原体污染情况，中图为支原体轻度污染情况，箭头所指为微粒状的一些支原体，右图为支原体重度污染情况，可见大量微粒状的支原体。
- 如果发现有支原体污染，建议更换无污染的细胞进行培养。如果有必要去除支原体，可以使用Mycoplasma Removal Agent、cyprofloxacin或BM-Cyclin去除支原体污染。
- 本试剂盒如用于六孔板样品检测，至少可以检测100个样品。

包装清单:

产品编号	产品名称	包装
C0296-1	固定液	100ml
C0296-2	Hoechst染色液	10ml
C0296-3	抗荧光淬灭封片液	10ml
—	说明书	1份

保存条件:

4°C保存，一年有效，其中Hoechst染色液需避光保存。

注意事项:

- Hoechst染色试剂对人体有害，操作时请小心，并注意有效防护以避免直接接触人体或吸入体内。
- 固定液含有乙酸，有刺激性气味，宜在通风橱内进行固定操作。
- 荧光染料都存在淬灭的问题，建议染色后尽量当天完成检测。
- 检测支原体前最好用不含抗生素的培养液培养2-3代，这样更容易检测出支原体，因为一些抗生素可以抑制支原体生长。
- 需自备PBS。
- 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. 细胞样品的准备:
   a. 对于贴壁细胞，在六孔板或其它多孔板内或盖玻片上培养细胞至50%-80%满。细胞培养过满会导致很难判断是否存在支原体污染。

b．对于悬浮细胞，离心沉淀细胞，取少量细胞做细胞涂片，空气中充分晾干。

2．用PBS按照1:10稀释Hoechst染色液(1体积Hoechst染色液加入9体积PBS)。
稀释后的Hoechst染色液宜在24小时内使用。

3．加适量固定液固定10-20分钟。
对于六孔板的一个孔，加入1ml固定液。确保固定液充分覆盖样品，固定前切勿对细胞进行洗涤。另外对于贴壁细胞，固定前需去除培养液。

4．去除固定液，空气中晾干。

5．加适量10倍稀释的Hoechst染色液室温染色10-30分钟。
对于六孔板的一个孔，加入1ml染色液。确保染色液充分覆盖样品，染色时注意避光。可以用铝箔纸避光。

6．去除染色液，空气中晾干。

7．滴加试剂盒中提供的抗荧光淬灭封片液，封片后荧光显微镜下观察蓝色荧光。
荧光显微镜观察时需400倍或1000倍放大观察(需使用油镜)。参考上图判断支原体污染情况。没有支原体污染或其它原核生物污染的细胞样品，仅观察到细胞核的蓝色荧光，线粒体DNA不会被染色。有支原体污染的细胞样品可以观察到细胞核周围微粒状或丝状蓝色荧光。支原体污染严重的细胞可以观察到大量微粒状或丝状蓝色荧光。有细菌、酵母或霉菌污染的情况虽然Hoechst染色也会呈阳性，但细菌、酵母或霉菌污染在光学显微镜下可以观察到，而支原体观察不到，由此可以初步区分是支原体还是其它微生物。如有必要进一步鉴定，可以通过把支原体接种培养和RT-PCR等方法进行进一步检测。

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