

碱性磷酸酶检测试剂盒

产品编号	产品名称	包装
P0321S	碱性磷酸酶检测试剂盒	100次
P0321M	碱性磷酸酶检测试剂盒	500次

产品简介:

- 碧云天生产的碱性磷酸酶检测试剂盒(Alkaline Phosphatase Assay Kit)是一种用于快速、便捷地检测细胞或组织样品的裂解或匀浆产物的上清液、血清、血浆、尿液等样品中内源性的碱性磷酸酶活性的试剂盒。
- 碱性磷酸酶(Alkaline Phosphatase, AP/ALP/AKP/ALKP/ALPase/Alk Phos)也称碱性磷酸酯酶(EC 3.1.3.1), 可以在碱性条件下催化磷酸酯键的水解。哺乳动物中, 肝脏、胆管、肾脏、骨头和胎盘中的碱性磷酸酶活性比较高。常见的碱性磷酸酶包括肠道碱性磷酸酶(alkaline phosphatase, intestinal, ALPI)、非组织特异性碱性磷酸酶(alkaline phosphatase, tissue-nonspecific isozyme, ALPL)和胎盘碱性磷酸酶(alkaline phosphatase, placental type, 也称placental alkaline phosphatase, PLAP)。常见的小牛肠碱性磷酸酶(Calf Intestinal Alkaline Phosphatase, CIAP/CIP)被广泛用于二抗等的标记最终用于蛋白和核酸等的检测, 也常用于DNA或RNA 5'和3'末端的去磷酸化(去单磷酸化), 特别是质粒的5'末端去磷酸化以避免质粒自连等。
- 干细胞, 如iPS中, 碱性磷酸酶的活性很高, 常被用作iPS成功诱导的标志。另外, 分化的结肠癌细胞中碱性磷酸酶的活性也会显著升高, 被当作结肠癌细胞分化程度定性和定量的指标。此外, 血清中碱性磷酸酯酶的升高, 被称作高碱性磷酸酶血症(hyperalkalinephosphatasemia), 被认为和恶性胆管阻塞(malignant biliary obstruction)、原发性胆管硬化(primary biliary cirrhosis)、原发性硬化胆管炎(primary sclerosing cholangitis)、肝淋巴瘤(hepatic lymphoma)和肝肉瘤(hepatic sarcoidosis)等肝胆疾病密切相关。血清中碱性磷酸酶活性升高也和骨头生成密切相关, 因为碱性磷酸酶是成骨细胞的副产物。血清中碱性磷酸酶活性过低也和一些疾病相关。儿童和孕妇血清中的碱性磷酸酶活性较普通人高一些。血清中碱性磷酸酶活性范围在20-140U/L。
- 除胎盘碱性磷酸酶(alkaline phosphatase placental isoform)以外, 其它的内源性碱性磷酸酶加热后易失活。
- 本试剂盒可以检测细胞或组织样品的裂解或匀浆产物的上清液、血浆、血清、尿液或纯化的酶样品等中的碱性磷酸酶活性。
- Para-nitrophenyl phosphate (pNPP)是一种常用的磷酸酶显色底物, 在碱性条件下, 可在碱性磷酸酶作用下生成 para-nitrophenol. para-nitrophenol (*p*-nitrophenol)在碱性条件下, 呈黄色产物, 可以在400-415nm检测吸光度。产物黄色越深, 说明碱性磷酸酶活性越高, 反之则酶活性越低。据此通过比色分析就可以计算出碱性磷酸酶活性水平。
- 包括标准品和空白对照, 本试剂盒P0321S包装共可进行100个样品的检测, P0321M包装共可进行500个样品的检测。

包装清单:

产品编号	产品名称	包装
P0321S-1	检测缓冲液	15ml
P0321S-2	显色底物	2管
P0321S-3	<i>p</i> -nitrophenol溶液(10mM)	0.1ml
P0321S-4	反应终止液	12ml
—	说明书	1份

产品编号	产品名称	包装
P0321M-1	检测缓冲液	65ml
P0321M-2	显色底物	5管
P0321M-3	<i>p</i> -nitrophenol溶液(10mM)	0.5ml
P0321M-4	反应终止液	60ml
—	说明书	1份

保存条件:

-20°C保存, 一年有效。其中显色底物和*p*-nitrophenol溶液需避光保存。

注意事项:

- 如果希望进行酶活性的绝对定量, 进行酶反应时必须注意精确计时。此时推荐采用孵育30分钟等较长的时间, 以减小操作过程中

的时间误差。同时如果样品中酶活性较高，则可以预先适当稀释样品。

- 样品溶液中须避免出现EDTA、氟离子、柠檬酸盐等碱性磷酸酶的抑制剂。
- 检测缓冲液和*p*-nitrophenol溶液对人体有害，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。反应终止液有腐蚀性，操作时请小心，并注意有效防护以避免直接接触人体或腐蚀其他物品。
- 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. 试剂准备：将所有试剂取出，恢复至室温使用。
 - a. 显色底物溶液：对于P0321S包装，请取一管显色底物，溶解于2.5ml的检测缓冲液中(可先用1ml检测缓冲液进行溶解，充分溶解和混匀后，转移至15ml离心管，再加入1.5ml检测缓冲液)，充分溶解和混匀，冰上放置。对于P0321M包装，请取一管显色底物，溶解于5ml的检测缓冲液中(可先用2ml检测缓冲液进行溶解，充分溶解和混匀后，转移至15ml离心管，再加入3ml检测缓冲液)，充分溶解和混匀，冰上放置。新鲜配制的显色底物溶液需在6小时内使用。
 - b. 标准品工作液：取10 μ l *p*-nitrophenol溶液(10mM)，用检测缓冲液稀释至0.2ml，最终浓度为0.5mM。
2. 样品准备：
 - a. 细胞或组织裂解液的准备：采用适当细胞或组织裂解液裂解细胞或组织，建议使用碧云天的P0013J Western及IP细胞裂解液(无抑制剂)裂解相关样品。如果有必要需进行适当匀浆，随后离心取上清，用于碱性磷酸酶的检测。注意：裂解液中不能含有磷酸酶抑制剂。样品可以-80 $^{\circ}$ C冻存，但需避免反复冻融。
 - b. 血浆、血清和尿液的准备：血浆和血清按照常规方法制备后可以直接用于本试剂盒的测定，但为了消除样品本身颜色的干扰，需设置加了血浆或血清但不加底物的对照。血浆制备时不能用含EDTA和柠檬酸盐的抗凝管。尿液通常也可以直接用于测定。上述样品可以-80 $^{\circ}$ C冻存，但需避免反复冻融。
 - c. 样品的稀释：如果样品中含有较高活性的碱性磷酸酶，可以使用原有的裂解液或PBS等进行稀释，也可以采用试剂盒中的检测缓冲液进行稀释。如果使用试剂盒中提供的检测缓冲液进行稀释，需注意保留足够的检测缓冲液用于试剂盒的检测过程。
3. 参考下表使用96孔板设置空白对照孔、标准品孔和样品孔。标准品的用量分别为4、8、16、24、32和40微升，样品通常可以直接加50微升。如果样品中的碱性磷酸酶活性过高，可以减少样品用量或适当稀释后再进行测定。

	空白对照(Blank)	标准品(Standard)	样品(Sample)
检测缓冲液	50 μ l	(100-x) μ l	(50-y) μ l
显色底物	50 μ l	—	50 μ l
样品	—	—	y μ l
标准品工作液	—	x μ l	—

4. 用枪头轻轻吹打混匀，也可借助摇床进行混匀。
5. 37 $^{\circ}$ C孵育5-10分钟。(说明：待测样品中碱性磷酸酶活性较低时，可适当延长孵育时间至30分钟)
6. 每孔加入100 μ l反应终止液终止反应。此时，标准品或有碱性磷酸酶活性的孔会呈现不同深浅的黄色。
7. 在405nm测定吸光度。如果不能测定405nm，也可以在400-415nm范围内检测吸光度。如果不能立即测定，可以在数小时内完成测定，所显现的黄色在数小时内稳定。
8. 碱性磷酸酶活性单位的定义：在pH9.8的diethanolamine(DEA)缓冲液中，37 $^{\circ}$ C条件下，每分钟水解para-nitrophenyl phosphate显色底物产生1微摩尔*p*-nitrophenol所需的碱性磷酸酶的量定义为一个酶活力单位，也被称作一个DEA酶活力单位。在pH9.6的甘氨酸缓冲液中，25 $^{\circ}$ C条件下，每分钟水解para-nitrophenyl phosphate显色底物产生1微摩尔*p*-nitrophenol所需的碱性磷酸酶的量定义为一个酶活力单位，也被称作一个Glycine酶活力单位。一个Glycine酶活力单位约相当于3个DEA酶活力单位。本试剂盒测定的是DEA酶活力单位。
9. 根据酶活性定义，计算出样品中的碱性磷酸酶活性。

相关产品：

产品编号	产品名称	包装
P0013J	Western及IP细胞裂解液(无抑制剂)	100ml
P0321S	碱性磷酸酶检测试剂盒	100次
P0321M	碱性磷酸酶检测试剂盒	500次
P0326	酸性磷酸酶检测试剂盒	120次
P0329	胎盘碱性磷酸酶检测试剂盒	100次
P0332	抗酒石酸酸性磷酸酶检测试剂盒	120次
P0335	抗氟离子酸性磷酸酶检测试剂盒	120次

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