Protein A+G Agarose

产品编号	产品名称	包装
P2012	Protein A+G Agarose	2ml

产品简介:

- 本Protein A+G Agarose为进口分装，主要用于免疫沉淀(Immunoprecipitation, IP)或免疫共沉淀(Co-IP)，也可以用于抗体的纯化。
- Protein A+G Agarose适合于免疫沉淀所有Protein A Agarose和Protein G Agarose单独可以免疫沉淀的抗体，包括mouse IgG_1，IgG_{2a}, IgG_{2b}, IgG_3, IgA, rat IgG_1, IgG_{2a}, IgG_{2b}, IgG_{2c}, rabbit IgG, rabbit and goat polyclonal Abs，以及human IgG_1, IgG_2, IgG_3和IgG_4。
- Protein A和Protein G都共价交联到4% agarose beads上，2ml Protein A+G Agarose中共含有约2mg重组的Protein A+G。2 ml Protein A+G Agarose共可以结合约14mg human IgG。
- Protein A+G Agarose配制在TBS溶液中，2ml中共含有0.5ml Agarose beads。
- 本Protein A+G Agarose如果用于常规的免疫沉淀，可以免疫沉淀100次。

包装清单:

产品编号	产品名称	包装
P2012	Protein A+G Agarose	1ml/管，共2管
—	说明书	1份

保存条件:

4℃保存，一年有效。

注意事项:

- 请勿冷冻保存本产品。
- Protein A+G Agarose使用前一定要充分重悬，即充分颠倒若干次使混合均匀。
- 从蛋白样品收集开始，所有步骤中蛋白样品都必须在4℃或冰上操作。
- 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. 免疫沉淀(Immunoprecipitation, IP):

A. 蛋白样品的准备:

A1. 对于10厘米细胞培养皿中的贴壁细胞，吸除细胞培养液，PBS洗涤一次，然后加入500微升至2毫升细胞裂解液裂解细胞。可以使用碧云天生产的Western及IP细胞裂解液(P0013)或各种RIPA裂解液(P0013B、P0013C、P0013D或P0013E)等进行细胞的裂解。

A2. 对于组织样品参考贴壁细胞使用裂解液的比例进行裂解。

A3. 对于悬浮细胞，离心收集细胞后，PBS洗涤一次，然后参考贴壁细胞的裂解方法进行裂解。

注：详细的裂解方法参考不同裂解液的详细使用方法。对于不同的培养器材，参考10厘米培养皿的裂解液的用量进行裂解。如果裂解获得的蛋白样品浓度过高，可以用裂解液或PBS适当稀释，如果蛋白样品浓度过低，在以后的裂解过程中宜适当减少裂解液的用量。

B. 去除非特异性结合**(可选做)**:

B1. 取200微升至1毫升蛋白样品，蛋白量约为200微克至1毫克，加入约1微克和免疫沉淀时使用的IgG种属相同的普通IgG和20微升充分重悬的Protein A+G Agarose，4℃缓慢摇动30分钟至2小时。

B2. 2500rpm(约1000g)离心5分钟，取上清用于后续的免疫沉淀。

注：所谓种属相同的IgG是指，例如后续免疫沉淀时用的是小鼠IgG，则在本步骤中可以加入normal mouse IgG，如无normal IgG可以加入其它不影响后续检测的其它mouse IgG类型的抗体。通过和normal IgG和Protein A+G Agarose的孵育，可以充分降低非特异性的结合，降低背景。

C. 免疫沉淀:

C1. 加入0.2-2微克用于免疫沉淀的一抗，4℃缓慢摇动过夜。

C2. 再加入20微升充分重悬的Protein A+G Agarose，4℃缓慢摇动1-3个小时。(为方便后续的洗涤操作可以把加入充分重悬的Protein A+G Agarose的量调整为40微升。)

C3. 2500rpm(约1000g)离心5分钟，或瞬时高速离心，小心吸除上清，注意宁可留下少量上清也不能吸掉Protein A+G Agarose。

C4. 用准备蛋白样品时的裂解液或PBS洗涤沉淀5次，裂解液或PBS的用量每次为0.5-1毫升。洗涤时离心条件和吸除上清的要求同上面的步骤C3。

C5. 完成最后一次洗涤后，去除上清，加入20-40微升1XSDS-PAGE电泳上样缓冲液Vortex重悬沉淀，瞬时高速离心把样品离心至管底。

C6. 100℃或沸水浴处理3-5分钟，取部分或全部样品用于SDS-PAGE电泳，暂时不用的样品可以-20℃保存。

2. 免疫共沉淀：

参考免疫沉淀的方法进行，但免疫共沉淀(co-IP)通常必须使用未经冻存的新鲜蛋白样品。普通的免疫沉淀虽然可以使用冻存的蛋白样品，但也宜用新鲜的蛋白样品为佳。

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