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# Protein A+G Agarose (Fast Flow, 进口分装)

| 产品编号 | 产品名称 | 包装 |
|---|---|---|
| P2012 | Protein A+G Agarose (Fast Flow, 进口分装) | 2ml |

## 产品简介：

- 本Protein A+G Agarose (Fast Flow)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 (Fast Flow)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15mg human IgG。推荐的线性流速(Linear flow rate)为 50-300cm/h。
- 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 (Fast Flow, 进口分装) | 1ml×2 |
| — | 说明书 | 1份 |

## 保存条件：

4ºC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4ºC或冰上操作。
- 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. 免疫沉淀(Immunoprecipitation, IP)：**

a. 蛋白样品的准备：

(a) 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。

(b) 对于组织样品参考贴壁细胞使用裂解液的比例进行裂解。

(c) 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)**：

(a) 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，4ºC缓慢摇动30分钟至2小时。

(b) 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：

(a) 加入0.2-2微克用于免疫沉淀的一抗，4ºC缓慢摇动过夜。

(b) 再加入20微升充分重悬的Protein A+G Agarose，4ºC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。)

(c) 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。

(d) 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c(c)。

(e) 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。

(f) 100ºC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-20ºC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。

## 相关产品：

| 产品编号 | 产品名称 | 包装 |
|---|---|---|
| P2006 | Protein A Agarose (Fast Flow, 进口分装) | 2ml |
| P2009 | Protein G Agarose (Fast Flow, 进口分装) | 2ml |
| P2012 | Protein A+G Agarose (Fast Flow, 进口分装) | 2ml |

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