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ChIP Assay Kit

产品编号	产品名称	包装
P2078	ChIP Assay Kit	22次

产品简介：

- ChIP Assay Kit即Chromatin Immunoprecipitation (ChIP) Assay Kit，也称染色质免疫沉淀检测试剂盒或ChIP检测试剂盒，用于通过免疫沉淀来沉淀和目标蛋白结合的染色质片段，最后通过PCR或Southern等方法来检测沉淀的染色质片段的试剂盒。通常用于检测特定的转录因子或组蛋白等基因组DNA结合蛋白是否和预期的特定基因组DNA序列在同一复合物中。
- 通过ChIP检测可以获得在体的(In Vivo)目标蛋白和预期基因组DNA片段是否在同一复合物中的结论。EMSA，也称gel shift获得的结果是体外的(In Vitro)目标蛋白和预期基因组DNA片段的结合结果，可以推断细胞内也发生类似的结合，但并不代表该情况在细胞内也真实发生。而ChIP的检测结果则可明确说明这种结合在细胞内是真实发生的。
- 本ChIP Assay Kit采用了Protein A+G Agarose，比Protein A Agarose或Protein G Agarose适合于免疫沉淀更多种类的抗体，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。
- 本试剂盒中经过Salmon Sperm DNA预饱和的Protein A+G Agarose和目的基因组DNA的非特异性结合大大下降。
- 提供了预混合的对照引物(Control Primers)。可用于扩增human GAPDH的部分相应序列，引物序列为：5’-TACTAGCGGTTTTACGGGCG-3’；5’-TCGAACAGGAGGAGCAGAGAGCGA-3’。
- 本ChIP Assay Kit如果用于常规的染色质免疫沉淀，共可以免疫沉淀22个样品。

包装清单：

产品编号	产品名称	包装
P2078-1	Protein A+G Agarose/Salmon Sperm DNA	3ml
P2078-2	Glycine Solution (10X)	30ml
P2078-3	ChIP Dilution Buffer	48ml
P2078-4	Low Salt Immune Complex Wash Buffer	24ml
P2078-5	High Salt Immune Complex Wash Buffer	24ml
P2078-6	LiCl Immune Complex Wash Buffer	24ml
P2078-7	TE Buffer	48ml
P2078-8	0.5M EDTA	250μl
P2078-9	5M NaCl	500μl
P2078-10	1M Tris, pH 6.5	500μl
P2078-11	SDS Lysis Buffer	10ml
P2078-12	Control Primers (5μM each)	0.1ml
—	说明书	1份

保存条件：

4°C保存，一年有效。

注意事项：

- 请勿冷冻保存P2078-1 Protein A+G Agarose/Salmon Sperm DNA。除P2078-1外，其它溶液可以-20°C冷冻以保存更长时间。
- 需自备用于ChIP的一抗，37%甲醛，PBS，PMSF，Elutioin (1% SDS, 0.1M $NaHCO_3$)，蛋白酶K，Glycogen或tRNA，Tris平衡苯酚，氯仿，95%乙醇，70%乙醇，3M NaAc (pH5.2)以及细胞刮子或细胞铲子。PMSF(ST506)，蛋白酶K(ST532/ST533)，Glycogen(D0812)和3M NaAc pH5.2(ST351)等可以向碧云天订购。
- 需自备超声样品处理仪(sonicator)，也称超声粉碎机或超声细胞粉碎机。
- 使用甲醛时请在通风橱中进行操作。
- 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. 样品超声处理条件的优化：

a. 准备适量冰浴预冷的PBS，以及100mM PMSF。将SDS Lysis Buffer适当温浴，使其中的SDS充分溶解，并混匀。

b. 将细胞培养于10cm细胞培养皿中，细胞培养液的用量为10 ml。在预期发生目的蛋白和基因组DNA结合的时间点，直接在细胞培养液中加入适量甲醛，轻轻混匀，至最终浓度为1%。随即在37°C孵育10分钟，以交联目的蛋白和相应的基因组DNA。例如，对于常规的每个10cm细胞培养皿中加入10 ml细胞培养液的情况，需加入270微升37%甲醛。请注意尽量使用高质量的在有效使用期限内的甲醛。细胞也可以培养于6cm细胞培养皿中，相关溶液的用量需按照比例进行相应调整。

c. 加入1.1ml Glycine Solution (10X)，轻轻混匀。室温放置5分钟。

d. 将有细胞样品的培养皿置于冰浴上。吸尽含甲醛和glycine的培养液，尽量保持没有液体残留。

e. 在上述室温放置5分钟期间，用冰浴预冷的PBS稀释100mM PMSF至1mM，即配制成冰浴预冷的含1mM PMSF的PBS。PMSF水性溶液一定要新鲜配制，其在水相中的半衰期约为30分钟。

f. 加入5-10ml冰浴预冷的含1mM PMSF的PBS，洗涤细胞，吸尽液体，尽量保持没有液体残留。

g. 再加入5-10ml含冰浴预冷的1mM PMSF的PBS，进一步洗涤细胞，吸尽液体，尽量保持没有液体残留。

h. 加入1ml冰浴预冷的含1mM PMSF的PBS，用细胞刮子刮下细胞，收集至离心管中。如果细胞可以用枪吹打下来，也可以用枪吹打。对细胞进行计数，分装成每管大约100万细胞。

i. 4°C，800-1000g离心1-2分钟，以充分沉淀细胞。如果发现沉淀不充分，可以适当延长离心时间。吸尽上清，尽量减少液体残留。

j. 配制适量含有1mM PMSF的SDS Lysis Buffer。上一步骤的100万细胞沉淀用0.2ml含有1mM PMSF的SDS Lysis Buffer重悬。

k. 在冰浴上孵育10分钟，以充分裂解细胞。

l. 超声处理，以剪切基因组DNA，使DNA大部分断裂成200-1000bp大小，如果能把大部分控制在400-800bp则更佳。超声过程中请一定注意要保持样品处于冰浴中，并且处于较低温度。超声剪切的效果在后续去交联后可以用常规的DNA琼脂糖凝胶电泳检测。超声处理的条件通常可以设置为每次超声10秒，停10秒，共5-30次左右，实际功率为10-40W，采用2-3mm超声头。不同的超声处理仪器的具体设置可能会不太一样，摸索超声条件时，可以先固定其他条件，先确定每次超声和暂停多长时间(优先推荐尝试每次超声10秒停10秒或者超声10秒停20秒)不会导致明显发热，且无泡沫产生，然后摸索不同的超声次数(例如5、10、20或30次)，通常实际功率越大，总超声时间越少。直至找到比较合适的超声次数可以使大部分基因组DNA断裂成200-1000bp大小。需要注意的是每次的超声体积和细胞种类及用量宜固定，否则就不能使用一个相对比较固定的超声条件用于后续实验。

   注：在对超声后基因组DNA大小进行检测时，如果采用琼脂糖凝胶中添加NA-Red、NA-Green、Gel-Red或Gel-Green等安全染料或使用含该类安全染料的DNA上样缓冲液的方式，由于电泳时SDS会与此类染料结合形成异常条带，条带通常在500-1000bp左右，因此会对超声后基因组DNA大小的判断造成一定的影响。建议采用“电泳完毕后对琼脂糖凝胶染色”的方式进行条带大小的检测，使用该方法不会有异常条带出现，不影响对超声后基因组DNA大小的判断，而且条带大小更准确。

m. 在0.2ml经过超声处理的样品中加入8微升5M NaCl，混匀。65°C加热4小时，以去除蛋白和基因组DNA之间的交联。

n. 加入等体积的Tris平衡苯酚，vortex剧烈混匀，随后4°C，12000g左右离心5分钟。吸取上清至另一离心管中。

o. 加入等体积氯仿，vortex剧烈混匀，随后4°C，12000g左右离心5分钟。吸取上清至另一离心管中。
   说明：上述步骤1N和1O的酚氯仿抽提可以使用DNA纯化试剂盒进行操作。例如碧云天的PCR/DNA纯化试剂盒(D0033)。

p. 取少量通过酚氯仿抽提或DNA纯化试剂盒获得的液体，对于酚氯仿抽提产物可以取5-10微升，对于DNA纯化试剂盒纯化产物可以取2-5微升，进行琼脂糖凝胶电泳，观察超声处理对于基因组DNA的剪切效果。

2. 染色质免疫沉淀：

a. 在对样品超声处理条件进行优化后，对于待检测样品按照步骤1A-1K进行操作，并参考步骤1L进行超声处理。

b. 随后对于经过超声处理的样品在4°C，12000-14000g离心5分钟。取上清(约0.2ml)至一2ml离心管中，置于冰浴。

c. 配制适量含有1mM PMSF的ChIP Dilution Buffer。加入1.8ml含有1mM PMSF的ChIP Dilution Buffer以稀释经过超声处理的样品，使最终体积为2毫升。

d. 取出20微升样品作为Input用于后续检测。其余近2ml样品加入70微升Protein A+G Agarose/Salmon Sperm DNA(其中约35微升为沉淀，35微升为液体)，在4°C缓慢转动或摆动混匀30分钟。此步骤的目的是减少Protein A+G Agarose/Salmon Sperm DNA和目的蛋白或目的DNA序列的非特异性结合。

e. 4°C，1000g左右离心1分钟，将上清转移至一个新的2毫升离心管中。

f. 加入适量一抗，一抗的用量可以参考抗体的说明书。如果抗体的说明中未给出用于ChIP的稀释比例，可以参考普通的免疫沉淀的稀释比例。通常一抗的用量为0.5-1微克。4°C缓慢转动或摆动混匀过夜。可以不加抗体作为阴性对照，或用无关的抗体作为阴性对照，同时可以用没有细胞样品的溶液作为空白对照。

g. 加入60微升Protein A+G Agarose/Salmon Sperm DNA(其中约30微升为沉淀，30微升为液体)，在4°C缓慢转动或摆动混匀60分钟，以沉淀一抗识别的蛋白或相应的复合物。

h. 4°C，1000g左右离心1分钟。非常小心地去除液体，切勿触及沉淀。随后依次用如下溶液对沉淀进行洗涤，每次洗涤液的用量为1ml，每次在4°C缓慢转动或摆动洗涤3-5分钟，随后4°C，1000g左右离心1分钟。非常小心地去除液体，切勿触及沉淀。
   (a) Low Salt Immune Complex Wash Buffer洗涤一次。
   (b) High Salt Immune Complex Wash Buffer洗涤一次。
   (c) LiCl Immune Complex Wash Buffer洗涤一次。
   (d) TE Buffer洗涤两次。

说明：完成上述所有洗涤步骤后所获得的沉淀即可用于PCR扩增目的基因序列或用Southern检测目的基因序列，或者用于Western检测等。

3. **PCR扩增目的基因序列(**如果ChIP产物用于检测目的基因序列**)：**
   a. 新鲜配制适量Elution buffer (1% SDS, 0.1M $NaHCO_3$)。
   b. 完成步骤2H后，即完成所有洗涤步骤后，加入250微升Elution buffer。Vortex混匀，室温转动或摆动继续洗脱3-5分钟。
   c. 1000g左右离心1分钟，将上清转移到一新的离心管中。沉淀中再加入250微升Elution buffer。Vortex混匀，室温转动或摆动继续洗脱3-5分钟。
   d. 1000g左右离心1分钟，取出上清。和上一步骤，即步骤3C中获得的上清合并。共计约500微升上清。
   e. 在500微升上清中加入20微升5M NaCl，混匀。65°C加热4小时，以去除蛋白和基因组DNA之间的交联。对于步骤2D获得的作为Input的20微升样品，加入1微升5M NaCl，混匀，65°C加热4小时，同样用于去除蛋白和基因组DNA之间的交联。此步骤完成后可以继续进行后续步骤，也可以先-20°C冻存，第二天继续后续步骤。
   说明：此时的样品已经可以用于PCR，可以尝试使用1、2、5或10微升样品作为模板用于PCR检测目的基因。此时PCR的效果和可能被沉淀下来的DNA的量，以及整个PCR扩增体系是否容易扩增目的基因有关。如果发现PCR效果欠佳，可以考虑通过后续的纯化步骤，纯化并浓缩样品，然后再进行PCR检测。
   注意：通常情况下，推荐进行后续纯化后再进行PCR检测，而Input通常不必进行后续纯化步骤。
   f. 在约520微升样品中加入10微升0.5M EDTA，20微升1M Tris pH 6.5和1微升20mg/ml 蛋白酶K。混匀后45°C孵育60分钟。
   g. 加入等体积Tris平衡苯酚，vortex剧烈混匀，随后4°C，12000g左右离心5分钟。吸取上清至另一离心管中。
   h. 加入等体积氯仿，vortex剧烈混匀，随后4°C，12000g左右离心5分钟。吸取上清至另一离心管中。
   i. 加入20微克glycogen或yeast tRNA，加入1/10体积的3M NaAc，pH5.2，再加入2.5倍体积无水乙醇。混匀后-70°C沉淀不少于1小时，或-20°C沉淀8小时以上。
   j. 4°C，12000-14000g离心10分钟，小心吸去大部分上清，切勿触及沉淀。
   k. 加入约1ml 70%乙醇洗涤沉淀。4°C，12000-14000g离心10分钟，小心吸去大部分上清，切勿接触沉淀。
   l. 4°C，12000-14000g离心1分钟，非常小心地吸除残留液体。
   m. 用少量TE或水重悬DNA沉淀，用于目的基因的PCR检测。用于PCR的引物最好能设计2组，可以用Input作为模板预先摸索出相应的PCR条件，并选择一组效果较好的引物用于最终的PCR检测。少数情况下，当PCR条带过弱时，可以采用nested PCR技术，进行两轮扩增。
   说明：步骤G至步骤M也可以采用适当的DNA纯化试剂盒纯化DNA，例如碧云天的PCR/DNA纯化试剂盒(D0033)。
4. **Western检测ChIP产物(如果ChIP产物用于Western检测)：**
   a. 接步骤2H，在完成所有的洗涤步骤后，加入25微升SDS-PAGE蛋白上样缓冲液(1X)。SDS-PAGE蛋白上样缓冲液(1X)可以用SDS-PAGE蛋白上样缓冲液(5X)用水稀释配制而成。沸水浴煮沸10分钟。
   b. 可以取10-20微升用于Western检测。

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