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EMSA/Gel-Shift 试剂盒

产品编号	产品名称	包装
GS002	EMSA/Gel-Shift 试剂盒	100次

产品简介：

- EMSA/Gel-Shift试剂盒(EMSA/Gel-Shift Kit)是用于EMSA(也称gel shift)研究的一个试剂盒。通过EMSA可以研究目的蛋白和特定的DNA序列的结合情况，从而可以研究细胞内一些转录因子的激活水平。本试剂盒提供了进行EMSA实验的探针标记、蛋白和DNA结合以及EMSA上样等的主要试剂，使EMSA实验变得简单方便。
- EMSA/Gel-Shift 结合缓冲液(5X)中含有poly(dI-dC)等有效成分。其中poly(dI-dC)的浓度经过优化，可以很好的消除蛋白和标记探针间的非特异性结合，同时又不会减弱目的转录因子和标记探针间的结合。
- 每个EMSA/Gel-Shift试剂盒足够标记10-20次探针，足够进行100个蛋白和探针的结合反应。

包装清单：

产品编号	产品名称	包装
GS002-1	T4 Polynucleotide Kinase	100U
GS002-2	T4 Polynucleotide Kinase Buffer (10X)	100μl
GS002-3	Nuclease-Free Water	1ml
GS002-4	探针标记终止液	100μl
GS002-5	5M 醋酸铵	600μl
GS002-6	EMSA/Gel-Shift结合缓冲液(5X)	200μl
GS002-7	EMSA/Gel-Shift上样缓冲液(蓝色，10X)	200μl
GS002-8	EMSA/Gel-Shift上样缓冲液(无色，10X)	200μl
GS002-9	TE	1ml/管，共2管
—	说明书	1份

保存条件：

-20°C保存，一年有效。

注意事项：

- 需自备待标记的EMSA探针，需自备用于探针标记的同位素，需自备EMSA胶配制的相关试剂。
- 细胞核蛋白的抽提可以使用碧云天生产的细胞核蛋白与细胞浆蛋白抽提试剂盒(P0028)。
- 如需做super-shift，需自备用于super-shift的抗体。
- 本实验涉及到同位素的操作，请严格按照同位素的相关管理条例进行操作。
- 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. 探针的标记：

1. 如下设置探针标记的反应体系：

待标记探针(1.75pmol/μl)	2μl
T4 Polynucleotide Kinase Buffer (10X)	1μl
Nuclease-Free Water	5μl
[γ-32P]ATP (3,000Ci/mmol at 10mCi/ml)	1μl
T4 Polynucleotide Kinase (5-10u/μl)	1μl
总体积	10μl

按照上述反应体系依次加入各种试剂，加入同位素后，Vortex混匀，再加入T4 Polynucleotide Kinase，混匀。

2. 使用水浴或 PCR 仪，37°C 反应 10 分钟。
3. 加入 1 微升探针标记终止液，混匀，终止探针标记反应。
4. 再加入 89 微升 TE，混匀。此时可以取少量探针用于检测标记的效率。通常标记的效率在 30%以上，即总放射性的 30%以上

标记到了探针上。为实验简便起见，通常不必测定探针的标记效率。

5. 标记好的探针最好立即使用，最长使用时间一般不宜超过 3 天。标记好的探针可以保存在-20°C。

2. 探针的纯化：

通常为实验简便起见，可以不必纯化标记好的探针。在有些时候，纯化后的探针会改善EMSA的电泳结果。如需纯化，可以按照如下步骤操作：

1. 对于100微升标记好的探针，加入1/4体积即25微升的5M醋酸铵，再加入2体积即200微升的无水乙醇，混匀。
2. 在-70°C至-80°C沉淀1小时，或在-20°C沉淀过夜。
3. 在4°C，12,000g-16,000g离心30分钟。小心去除上清，切不可触及沉淀。
4. 在4°C，12,000g-16,000g离心1分钟。小心吸去残余液体。微晾干沉淀，但不宜过分干燥。
5. 加入100微升TE, 完全溶解沉淀。标记好的探针最好立即使用, 最长使用时间一般不宜超过3天。标记好的探针可以保存在-20°C。

3. EMSA胶的配制：

1. 准备好倒胶的模具。可以使用常规的灌制蛋白电泳胶的模具，或其它适当的模具。最好选择可以灌制较薄胶的模具，以便于干胶等后续操作。为得到更好的结果，可以选择可灌制较大EMSA胶的模具。
2. 按照如下配方配制20毫升4%的聚丙烯酰胺凝胶(注意：使用29:1等不同比例的Acr/Bis对结果影响不大)。

TBE　buffer (10X)	1.0ml
重蒸水	16.2ml
39:1 acrylamide/bisacrylamide (40%,w/v)	2ml
80% 甘油	625μl
10% 过硫酸铵(ammonium persulfate)	150μl
TEMED	10μl

3. 按照上述次序加入各个溶液，加入TEMED前先混匀，加入TEMED后立即混匀，并马上加入到制胶的模具中。避免产生气泡，并加上梳齿。如果发现非常容易形成气泡，可以把一块制胶的玻璃板进行硅烷化处理。

4. EMSA结合反应：

1. 如下设置EMSA结合反应(预期的结果参见图1)：

阴性对照反应：

Nuclease-Free Water	7μl
EMSA/Gel-Shift 结合缓冲液(5X)	2μl
细胞核蛋白或纯化的转录因子	0μl
标记好的探针	1μl
总体积	10μl

样品反应：

Nuclease-Free Water	5μl
EMSA/Gel-Shift 结合缓冲液(5X)	2μl
细胞核蛋白或纯化的转录因子	2μl
标记好的探针	1μl
总体积	10μl

探针冷竞争反应：

Nuclease-Free Water	4μl
EMSA/Gel-Shift 结合缓冲液(5X)	2μl
细胞核蛋白或纯化的转录因子	2μl
未标记的探针	1μl
标记好的探针	1μl
总体积	10μl

突变探针的冷竞争反应：

Nuclease-Free Water	4μl
EMSA/Gel-Shift 结合缓冲液(5X)	2μl
细胞核蛋白或纯化的转录因子	2μl
未标记的突变探针	1μl
标记好的探针	1μl
总体积	10μl

Super-shift 反应：

Nuclease-Free Water	4μl
EMSA/Gel-Shift 结合缓冲液(5X)	2μl
细胞核蛋白或纯化的转录因子	2μl
目的蛋白特异抗体	1μl
标记好的探针	1μl
总体积	10μl

2. 按照上述顺序依次加入各种试剂，在加入标记好的探针前先混匀，并且室温（20-25°C）放置10分钟，从而消除可能发生的探针和蛋白的非特异性结合，或者让冷探针优先反应。然后加入标记好的探针，混匀，室温(20-25°C)放置20分钟。
3. 加入1微升EMSA/Gel-Shift上样缓冲液(无色，10X)，混匀后立即上样。注意：有些时候溴酚蓝会影响蛋白和DNA的结合，建议尽量使用无色的EMSA/Gel-Shift上样缓冲液。如果对于使用无色上样缓冲液在上样时感觉到无法上样，可以在无色上样缓冲液里面添加极少量的蓝色的上样缓冲液，至能观察到蓝颜色即可。

5. 电泳分析：

1. 用0.5XTBE作为电泳液。按照10V/厘米的电压预电泳10分钟。预电泳的时候如果有空余的上样孔，可以加入少量稀释好的1X的EMSA上样缓冲液(蓝色)，以观察电压是否正常进行。
2. 把混合了上样缓冲液的样品加入到上样孔内。在多余的某个上样孔内加入10微升稀释好的1X的EMSA/Gel-Shift上样缓冲液(蓝色)，用于观察电泳进行的情况。
3. 按照10V/厘米的电压电泳。确保胶的温度不超过30°C，如果温度升高，需要适当降低电压。电泳至EMSA/Gel-Shift上样缓冲

液中的蓝色染料溴酚蓝至胶的下缘1/4处，停止电泳。

4. 剪一片大小和EMSA胶大小相近或略大的比较厚实的滤纸。小心取下夹有EMSA胶的胶板，用吸水纸或普通草纸大致擦干胶板边缘的电压液。小心打开两块胶板中的上面一块(注：通常选择先移走硅烷化的那块玻璃板)，把滤纸从EMSA胶的一侧逐渐覆盖住整个EMSA胶，轻轻把滤纸和胶压紧。滤纸被胶微微浸湿后(大约不足1分钟)，轻轻揭起滤纸，这时EMSA胶会被滤纸一起揭起来。把滤纸侧向下，放平，在EMSA胶的上面覆盖一层保鲜膜，确保保鲜膜和胶之间没有气泡。
5. 在干胶仪器上干燥EMSA胶。然后用X光片压片检测，或用其它适当仪器设备检测。EMSA的典型分析结果可以参见下面的图1。

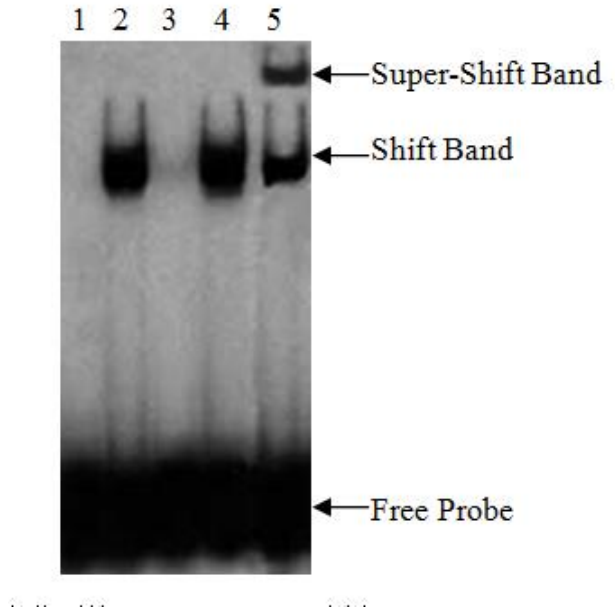

图1. 一个典型的EMSA/Gel-Shift分析图

1，**阴性对照反应**(标记探针)；**2，常规反应**(含激活的目的转录因子的核蛋白+标记探针)；**3，探针冷竞争反应**(含激活的目的转录因子的核蛋白+标记探针+标记探针100倍量的未标记探针)；**4，突变探针的冷竞争反应**(含激活的目的转录因子的核蛋白+标记探针+标记探针100倍量的未标记突变探针)；**5, Super-shift反应**(含激活的目的转录因子的核蛋白+标记探针+目的转录因子的特异抗体)。

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Version 2024.03.12