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化学发光法 EMSA 试剂盒

产品编号	产品名称	包装
GS009	化学发光法EMSA试剂盒	100次

产品简介：

- 化学发光法EMSA试剂盒(Chemiluminescent EMSA Kit)是一种通过Streptavidin-HRP及后续的BeyoECL Moon试剂来实现化学发光检测Biotin标记的EMSA探针的检测试剂盒。同时本试剂盒也提供了EMSA检测所需的结合缓冲液和上样缓冲液，及一些关键的相关试剂，可以实现非同位素的EMSA检测。
- 本试剂盒采用了高质量的Streptavidin-HRP Conjugate，HRP和Streptavidin共价交联的比例大于3，这样比采用Streptavidin和Biotin-HRP conjugate两种试剂进行检测要更方便，并且灵敏度更高。
- 本试剂盒采用了非特异性结合比avidin更低的strepatavidin，使检测结果背景更低灵敏度更高。
- 本试剂盒和碧云天的各种生物素标记EMSA探针可以配套使用。
- 本试剂盒没有提供生物素探针标记相关的试剂，其它特定的生物素标记EMSA探针的制备可以使用碧云天的EMSA探针生物素标记试剂盒(GS008)。
- 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。
- 本试剂盒可以用于100个蛋白和探针的结合反应，并足够检测至少10块有生物素标记EMSA探针的膜。

包装清单：

产品编号	产品名称	包装
GS009-1	EMSA/Gel-Shift结合缓冲液(5X)	200μl
GS009-2	EMSA/Gel-Shift上样缓冲液(无色，10X)	200μl
GS009-3	EMSA/Gel-Shift上样缓冲液(蓝色，10X)	200μl
GS009-4	BeyoECL Moon A液	55ml
GS009-5	BeyoECL Moon B液	55ml
GS009-6	Streptavidin-HRP Conjugate	100μl
GS009-7	封闭液	380ml
GS009-8	洗涤液(5X)	250ml
GS009-9	检测平衡液	250ml
—	说明书	1份

保存条件：

GS009-1至GS009-3和GS009-6在-20°C保存，其余可4°C保存。如果长期不用，整个试剂盒可-20°C保存，-20°C可以保存更长时间。

注意事项：

- 需自备带正电荷尼龙膜，以及凝胶电泳时所需的相关试剂。带正电荷尼龙膜(FFN10/FFN11/FFN13/FFN15)可以向碧云天订购。
- 如果需要使用更多的封闭液或洗涤液，可另外单独订购GS009B封闭液或GS009W洗涤液(5X)。
- BeyoECL Moon A液和B液均对人体有害，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。
- 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：

可以直接选购碧云天的生物素标记EMSA探针，或使用碧云天的EMSA探针生物素标记试剂盒(GS008)或其它合适的试剂盒进行EMSA探针的生物素标记。

2. 探针的纯化和检测：

碧云天的各种生物素标记EMSA探针都已经过纯化，可以直接使用；对于使用碧云天的EMSA探针生物素标记试剂盒(GS008)等试剂盒标记的EMSA探针，通常为实验简便起见，可以不必纯化标记好的探针。有些时候，纯化后的探针会改善EMSA的电泳结果。详细的探针纯化和探针的标记效率的检测请参考EMSA探针生物素标记试剂盒的相关说明。

3. EMSA胶的配制：

a. 准备好倒胶的模具。可以使用常规的制备蛋白电泳胶的模具(例如BioRad的常规用于蛋白电泳的制胶装置)，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。制胶前必须把制胶模具冲洗干净，需特别注意不能有SDS残留。

b. 按照如下配方配制20ml 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

c. 按照上述顺序依次加入各种试剂，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：

a. 如下设置EMSA结合反应：

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

b. 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。

c. 加入1μl 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，至可以观察到蓝颜色即可。

5. 电泳：

a. 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。

b. 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10μl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。

c. 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。

6. 转膜：

a. 取一和EMSA胶大小相近或略大的尼龙膜，剪角做好标记，用0.5XTBE浸泡至少10分钟。尼龙膜自始至终仅能使用镊子夹取，并且仅可夹取不可能接触样品的边角处。

b. 取两片和尼龙膜大小相近或略大的滤纸，用0.5XTBE浸湿。

c. 把浸泡过的尼龙膜放置在一片浸湿的滤纸上，注意避免尼龙膜和滤纸间产生气泡。

d. 非常小心地取出EMSA胶放置到尼龙膜上，注意确保胶和膜之间没有气泡。

e. 再把另外一片浸湿的滤纸放置到EMSA胶上，注意确保滤纸和胶之间没有气泡。

f. 采用Western时所使用的湿法电转膜装置或其它类似的电转膜装置，以0.5XTBE为转膜液，把EMSA胶上的探针、蛋白以及

探针和蛋白的复合物等转移到尼龙膜上。对于大小约为10x8x0.1cm的EMSA胶，用BioRad的常用的Western转膜装置，电转时可以设置为380mA(约100V)转膜30-60分钟。如果胶较厚，则需适当延长转膜时间。转膜时需保持转膜液的温度较低，通常可以把电转槽置于4°C冷库或置于冰浴或冰水浴中进行电转，这样可以确保低温。具体的电转膜方法请参考电转膜装置的使用说明。

g. 转膜完毕后，小心取出尼龙膜，样品面向上，放置在一干燥的滤纸上，轻轻吸掉下表面明显的液体。立即进入下一步的交联步骤，不可使膜干掉。

7．交联：

a. 用紫外交联仪(UV-light cross-linker)选择254nm紫外波长，120mJ/cm2，交联45-60秒。如果没有紫外交联仪可以使用普通的手提式紫外灯(例如碧云天的手提紫外检测仪(EUV002))，距离膜5-10厘米左右照射3-10分钟。也可以使用超净工作台内的紫外灯，距离膜5-10厘米左右照射3-15分钟。最佳的交联时间可以使用标准品自行摸索。

b. 交联完毕后，可以直接进入下一步检测；也可以用保鲜膜包裹后在室温干燥处存放3-5天，然后再进入下一步检测。

c. 如果检测结果发现交联效果不佳，甚至连free probe的条带都非常微弱，可以考虑在膜干燥后参考步骤A的条件再交联一次，以进一步改善交联效果。

8．化学发光法检测生物素标记的探针：

a. 37-50°C水浴溶解封闭液和洗涤液。

注意：封闭液和洗涤液必须完全溶解后方可使用，封闭液和洗涤液可以在室温至50°C之间使用，但必须确保这两种溶液中均无沉淀产生，在冬天需特别注意。

b. 取一合适的容器加入15ml封闭液，再放入交联过的含有样品的尼龙膜。在侧摆摇床或水平摇床上缓慢摇动15分钟。

c. 取7.5μl Streptavidin-HRP Conjugate加入到15ml封闭液中(1:2000稀释)，混匀备用。

d. 去除用于尼龙膜封闭的封闭液，加入上一步中配制的15ml含有Streptavidin-HRP Conjugate的封闭液。在侧摆摇床或水平摇床上缓慢摇动15分钟。

e. 取25ml洗涤液(5X)，加入100ml重蒸水或Milli-Q级纯水，混匀配制成125ml洗涤液。

f. 将尼龙膜转移至另一装有15-20ml洗涤液的容器内，漂洗1分钟。

g. 去除洗涤液，加入15-20ml洗涤液，在侧摆摇床或水平摇床缓慢上洗涤5分钟。

h. 重复步骤G 三次(共洗涤四次)，每次洗涤时间都约为5分钟。

i. 将尼龙膜转移至另一装有20-25ml检测平衡液的容器内，在侧摆摇床或水平摇床上缓慢摇动5分钟。

j. 取5ml BeyoECL Moon A液和5ml BeyoECL Moon B液混匀，配制成BeyoECL Moon工作液。注意：BeyoECL Moon工作液必须现配现用。说明：从本步骤起操作方法和注意事项同Western实验的荧光检测。

k. 取出尼龙膜，用吸水纸吸去过多液体。立即将膜的样品面向上，放置到处于水平桌面上的洁净容器内或保鲜膜上。

l. 在尼龙膜的表面小心加上步骤J配制好的共10ml BeyoECL Moon工作液，使工作液完全覆盖尼龙膜。室温放置2-3分钟。

m. 取出尼龙膜，用吸水纸吸去过多液体。将尼龙膜放在两片保鲜膜或其它适当的透光薄膜中间，并固定于压片暗盒(也称片夹)内。

n. 用X光片压片1-5分钟。可以先压片1分钟，立即显影定影，然后根据结果再调整压片时间；也可以直接分别压片30秒、1、3、5分钟或更长时间，然后一起显影定影观察结果。

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