proteintech®
Antibodies | ELISA kits | Proteins

www.ptglab.com

Dot Blot (For 68301-1-Ig only)

Protocol for DNA-dot blot (English version)

1. Extract DNA from the samples with extraction reagent (or kit) and formulate a proper final concentration between 0.05 μg/μLto 2 μg/μL. Dilute with PBS if the concentration is too high. Avoid contamination by DNase through the whole process (similarly hereinafter).

2. Directly dot the extracted DNA onto the nitrocellulose (NC) membrane. Mark the dot position and front side of the membrane. Appropriate amount of DNA for each dot is between 0.2–2 μg, and serial dilutions are recommended for the determination of the optimal amount at the first trial. Adjust each dot to the same volume by dilution with PBS if needed. In publications, nylon membrane is also used, where it is usually cross linked via UV irradiation after dotting. Take NC membrane as an example below.

3. Dry the membrane for 5–15 minutes in air, and block with 1% BSA (in PBST) solution for one hour. If methylene blue staining is applied as loading control, the dired membrane can be directly stained without blocking. Blocking will interfere the staining effect of methylene blue.

4. Briefly wash with PBS, PBST, or TBST once. Dilute the primary antibody with 1% BSA (in PBST) solution to an appropriate concentration. For Proteintech's 68301-1-Ig, 1 : 5000 dilution is recommended. Add the diluted primary antibody solution to the membrane, and incubate at room temperature for 1.5 hours. Ensure that the primary antibody solution completely covers the membrane. Avoid using milk for dilution of primary antibody in this experiment.

5. Wash with PBST for 4 times, 5 minutes per time. Discard the washing buffer, add the secondary antibody diluted in 1% BSA, and incubate at room temperature for 1.5 hours or at 37 ℃ for 1 hour. Ensure that the secondary antibody solution completely covers the membrane. Avoid using milk for dilution of secondary antibody in this experiment.

6. Wash in PBST for 4 times, 5 minutes per time. Completely discard the washing buffer and add ECL substrate for the luminescent development.

Protocol for DNA-dot blot (Chinese version)

1. 用 DNA 提取试剂（或试剂盒）提取 DNA，最终浓度不低于 0.05 μg/μL，不高于 2 μg/μL 为宜，如果过高可以用 PBS 稀释。注意全程防止DNAase 污染（下同）。

2. 将提好的 DNA 直接点在 NC 膜上，注意做好记号用于标记点的位置和膜的正反面，每个点点 0.2-2 μg DNA 为宜，第一次做建议做不同梯度以确定最佳条件。个点的体积尽量调成一样，可以用 PBS 调整稀释。也有文章报道用尼龙膜进行的，尼龙膜点完膜后一般会用紫外照射交联。以下以 NC 膜为例。

3. 膜静 5-15 分钟稍稍晾干后用 1 %BSA 封闭一小时（PBST 中）。如需要做亚甲基蓝作 loading control，则点完膜后稍稍晾干不做封闭就可以直接染膜。封闭会干扰亚甲基蓝染色效果。

4. 用 PBS 或 PBST 或 TBST 简单洗涤一次。将一抗用 1% BSA（PBST 中）稀释至合适浓度，如 Proteintech 的 68301-1-Ig 建议按 1：5000 稀释。向膜上加入稀释好的一抗，室温反应 1.5 小时。确保一抗能完全淹没膜。注意本实验中一抗不建议用牛奶稀释。

5. 用 PBST 洗涤 4 次，每次 5 分钟。弃去洗涤液后加入用 1% BSA 稀释好的二抗，室温反应 1.5 小时或 37 ℃反应 1 小时。确保二抗能完全淹没膜。注意本实验中二抗不建议用牛奶稀释。

6. 用 PBST 洗涤 4 次，每次 5 分钟。完全弃去洗涤液后加 ECL 底物显影。