Enhancer™ Dot-Blot Micro-Array System

(Cat. # 786-163)

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INTRODUCTION

The Enhancer™ Dot Blot/Micro-Arrays System allows application of 1 to 384 liquid samples on the membrane by capillary action at the point of application. The capillary-action application prevents protein and nucleic acid samples from spreading over a large membrane area. Thus, Enhancer™ System creates concentrated sample spots on the membrane and enhances the sensitivity. The device may be used for preparation of dot blots or multi-sample micro-arrays of up to 384 samples. Enhancer™ System is recommended for use with nitrocellulose and nylon membranes and not recommended for use with PVDF membranes (Patent Pending).

ITEM(S) SUPPLIED (Cat. # 786-163)

Description	Size
Tube-Strip Plate	1
Application Rack	1
Working Tray	1
Cover Plate	1
Sample Tube-Strips (For 96 sample tubes)	12 Strips (8tubes/strip)
Membrane Frames	2
Rubber Padding	1
Protein Binding Buffer	1ml
Forceps	1

ADDITIONAL ITEM(S) REQUIRED

- Membranes (12cm x 9cm) *(Cat. # 786-165NC and/or 786-165NY)*
- Sample application buffer & washing buffer
  ***NOTE**- Additional supply of Sample Tube-Strips (Cat. # 786-164) and Protein Binding Buffer (Cat. # 786-166) may be purchased separately.*

STORAGE CONDITIONS

The kit is shipped at ambient temp. Store Protein Binding Buffer at 4°C, all other items at room temperature.

SAMPLE PREPARATION

Protein Samples

- Protein samples should be prepared in buffers, containing 0.1-0.2M salts (e.g. NaCl), pH 7-8.
- The total protein applied on any spot should not exceed 2µg protein/spot, preferably, protein concentration of the sample should be under 1mg/ml. Alternatively, dilute the protein solution with an appropriate buffer.
- For the best results, we recommend mixing the protein sample with Protein Binding Buffer (i.e., 1µl Protein Binding Buffer per 4µl sample). The Protein Binding Buffer allows effective binding of protein to the nitrocellulose membrane and minimizes loss of the protein during washing steps. Protein binding buffer may be purchased separately (Cat. # 786-166).

Nucleic Acids

- Nucleic acid samples (DNA, RNA & Oligos) should be prepared in a buffer containing 0.1-0.2M salt (e.g. NaCl), pH 7-8.
- Alkaline denatured DNA must first be neutralized before application on the membrane.
- The total nucleic acid loaded on any spot should not exceed 2µg nucleic acid/spot, preferably, concentration of the sample should be under 1mg/ml. Alternatively, dilute the solution with an appropriate buffer.

PREPARATION BEFORE USE

Protein Binding Buffer may develop crystalline precipitate during cold storage. Allow the buffer to warm to room temperature or until crystals dissolve. Transfer 4-8µl of sample into a microfuge tube. Add 1µl Protein Binding Buffer per 4µl sample and mix well.

PROTOCOL: DOT BLOT PREPARATION

For application of 96 samples, use 12cm x 9cm membranes. For application of fewer than 96 samples, cut the membrane to an appropriate size. Mark the orientation of the membrane with a soft pencil.

The system is supplied with an Application Rack and a Working Tray for the preparation of samples.

1. Make sure the rubber padding is positioned in the Application Rack. Position the first frame (without marking) on top of the rubber padding. Position the membrane (orientation marked) in the area within the frame. Place the second frame (with grid marking) on top.
2. Position the Tube-Strip Plate in the working tray.
   NOTE *- The Tube-Strip Plate has orientation marked with etched circles "O" (1 to 4) at four corners of the plate. You may select and use any orientation of the plate and align with the A-1 position on the membrane frame. However, if you chose to re-apply a sample, you must use the same orientation of the Tube-Strip Plate used before and align with the A-1 position. By changing the orientation of the plate you can change the footprint of the sample tubes. Select appropriate number of Tube-Strips and position in the slots provided in the Tube-Strip Plate.*
   NOTE*- if you are not using the entire 96-samples, make sure that you have tubes at all 4-corners of the Tube-Strip Plate. If necessary load empty tubes at all four corner tube positions.*
3. Prepare the samples by adding 1-5µl sample into each tube. 1-5µl samples are appropriate for most applications. If necessary, up to 10µl samples may be applied on each spot. When applying more than 5µl samples, the diffusing buffer from adjacent samples may overlap. Since samples bind and are retained at the point of application, the overlapping buffer zone would not create problems.
   IMPORTANT*- Make sure the samples are free from particulate materials. Sample may be clarified by a centrifugation.*
   NOTE*- Load the samples into the tubes by touching the sidewall of the tubes. DO NOT LOAD THE SAMPLES INTO THE BOTTOM CENTER OF THE TUBE.*
   IMPORTANT*-The sample loaded on any spot should not exceed 2µg protein/spot or 2µg nucleic acid/spot.*
4. Remove the Tube-Strip Plate from the tray and gently position on top of the membrane secured in the Application Rack.
   NOTE*- Make sure the selected orientation of the plate is aligned with the A-1 position marking on the membrane frame. Place the Cover Plate on top of the Tube-Strips.*
5. Sample Application: After positioning the Cover Plate, gently tap the Cover Plate. This will allow the samples to slowly migrate into the bottom center of the tube. As soon as the samples reach the center bottom of the Tubes, the sample begins to diffuse into the membrane. Application time will depend on the type of membrane and the nature of the sample, generally, 1-5µl samples take 1-2 minutes. It may be

necessary to check the Tube-Strips; some samples may require a longer diffusion time. Simply remove strip to visually check diffusion. Replace Tube-Strips into Tube-Strip Plate if diffusion is incomplete.

6. After the samples have diffused into the membrane, remove the membrane from the Application Tray. Discard the Tube-Strips. The membrane is now ready for further processing.

PROTOCOL: MICRO ARRAY PREPARATION

Enhancer™ Dot Blot/Micro-Arrays may be used for application of up to 384 samples on a single sheet of membrane. The Tube-Strip Plate has orientation marked with etched circles "O" (1 to 4) at four corners of the plate. By changing the orientation of the Tube-Strip Plate you can change the footprint of the sample tubes on the membrane. Each orientation of the plate will allow application of 96 samples. By selecting the orientation 1 to 4 you can apply up to 384 samples (one set of 96 applications after another), where each spot/sample will be 3-4mm apart.

1. Select the orientation "O" of the Tube-Strip Plate and apply a set of 96 samples (as described above).
2. For application of another set of 96 samples, select orientation "O O" of the Tube-Strip Plate and repeat application of the samples.
3. For application of a third and a fourth set of 96 samples, select the orientation "OOO" and "OOOO", respectively, and repeat the sample applications.

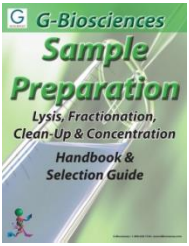
TROUBLESHOOTING:

If sample does not flow or diffuse into the membrane:

1. Tap the plate or individual tubes or strips a few more times to allow the sample to reach the bottom center of the tube.
2. Make sure the sample is not too concentrated, viscous or particulate. Diluting the sample will help the flow.
3. Replace the single tube with problem (a single tube may be cut & removed from the tube-strip). Transfer the sample into a new tube. Repeat the Sample Application protocol.

RELATED PRODUCTS

Download our Sample Preparation Handbook.



http://info.gbiosciences.com/complete-protein-sample-preparation-handbook

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