

Example Procedure for the Use of 3DNA[®] UltraAmp[™] Signal Amplification Reagents with FAST[®] Slide Nitrocellulose Microarrays from Schleicher & Schuell (S&S)

Other Materials Required or Recommended

- Microarray reader equipped to read Oyster[®]-650 fluorochromes
- S&S FAST Slide Nitrocellulose Array
- UltraAmp Anti-Biotin or Anti-FITC labeled with 50 Oyster-650 Dyes (Do not use UltraAmp reagents labeled with 350 or 900 Oyster-650 Dyes. Do not use UltraAmp reagents labeled with Oyster-550 dyes.)
- Cot-1 DNA, 1 μ g/ μ L (Invitrogen)
- Reagent Grade Deionized Water for preparing wash buffers (Recommended: VWR Cat No. RC9150-5)
- 1X Phosphate Buffer Saline (PBS), pH 7.0 – 7.5 + 0.02% Tween-20
- 1X PBS, pH 7.0 – 7.5, + 2.5% bovine serum albumin (BSA - fraction V or better quality)
- 2X PBS, pH 7.0 – 7.5, + 2.5% bovine serum albumin (BSA - fraction V or better quality)
- 24 x 50mm glass coverslips
- Glass slide staining jars (or equivalent)
- Primary Analyte (either labeled or unlabeled)
- Optional: Biotin Labeling Kit (Pierce)
- Optional: Fluorescein Labeling Kit (Pierce)
- Optional: Biotinylated secondary antibody
- Optional: FITC-labeled secondary antibody
- Optional: S&S rubber incubation chamber
- Optional: S&S Blocking Solution
- Optional: S&S Wash Buffer

Procedural Note:

Many of the steps in this procedure involve proceeding immediately to a next section of steps. When noted, do not allow the array to dry out between the steps.

Blocking the Array

1. Choose one of the following blocking solutions:
 - S&S Blocking Solution
 - 2X PBS + 2.5% BSA and 2 μ g/100 μ L Cot-1 DNA
2. Apply 100-150 μ L of the blocking solution to the array. Cover the array by affixing an adhesive backed seal to the S&S incubation chamber or applying a 24 x 50mm glass coverslip.
3. Incubate for 30 minutes at room temperature (20-25°C). During this incubation, prepare the primary analyte (see below).
4. Remove the blocking solution as follows:
 - If the S&S incubation chamber was used, remove the solution by pipette aspiration.
 - If a coverslip was used, hold the slide vertical for a few seconds to remove the coverslip and decant the solution.
5. Proceed immediately to the next step without allowing the membrane to dry.

Binding of Primary Analyte

The primary analyte (antigen or antibody) used in this procedure may be directly labeled with hapten (using biotin and/or fluorescein labeling kits from Pierce) or may be unlabeled. If unlabeled analyte is used for this step, a secondary reagent labeled with the appropriate hapten must be used.

1. Dilute the primary analyte to an appropriate concentration in 1X PBS + 2.5% BSA. Human or animal serum samples may be added undiluted if desired.
2. Add 150-200 μ L of the sample to the membrane array. Cover the array by affixing an adhesive backed seal to the S&S incubation chamber or applying a 24 x 50mm glass coverslip.
3. Incubate for 60 minutes at an appropriate temperature for the analyte (generally 15-37°C).

Washing the Array

1. Remove the primary analyte as follows:
 - If the S&S incubation chamber was used, remove the solution by pipette aspiration, or, remove the entire incubation chamber
 - If a coverslip was used, partially immerse the array in 1X PBS and float the coverslip off.
2. Fully immerse the array into a suitable container (a discarded pipette box lid works well) pre-filled with 25-50mL of 1X S&S Washing Buffer or 1X PBS + 0.02% Tween-20. Wash with vigorous agitation on an orbital shaker at room temperature for 5-10 minutes.
3. Repeat the washes twice more with fresh changes of washing buffer.
4. Before the last wash is complete, be ready to proceed to the next appropriate section (do not allow the array to dry):
If the primary analyte was unlabeled, proceed to **Binding of Secondary Labeled Antibody**.
If the primary analyte was hapten labeled, proceed to **3DNA UltraAmp Hybridization**.

Binding of Secondary Labeled Antibody

This procedure is for the binding of a hapten-labeled secondary antibody (or antigen) to a primary target. Use this procedure only if unlabeled primary analyte was used in the previous step.

1. Dilute the secondary antibody to an appropriate concentration in 1X PBS + 2.5% BSA.
Note: Biotinylated antibodies available from S&S may be pre-diluted and may not require any dilution.
2. Add 150-200 μ L of the diluted secondary detection reagent to the array. Cover the array by affixing an adhesive backed seal to the S&S incubation chamber or applying a 24 x 50mm glass coverslip.
3. Incubate for 60 minutes at room temperature (20-25°C).

Washing the Array

1. Remove excess Secondary Antibody as follows:
 - If the S&S incubation chamber was used, remove the entire incubation chamber.
 - If a coverslip was used, partially immerse the array in 1X PBS and float the coverslip off.
2. Fully immerse the array into a suitable container (a discarded pipette box lid works well) pre-filled with 25-50mL of 1X S&S Washing Buffer or 1X PBS + 0.02% Tween-20. Wash with vigorous agitation on an orbital shaker at room temperature for 5-10 minutes.
3. Repeat the washes twice with fresh changes of washing buffer, for a total of three washes. During the washes, prepare a **3DNA UltraAmp Mix** for each array (see below).
4. Immediately proceed to **3DNA UltraAmp Hybridization**. Do not allow the membrane to dry.

3DNA UltraAmp Hybridization

1. Prior to completing the wash steps, prepare a **3DNA UltraAmp Mix** for each array. Do not exceed 100 μ L as a final volume per array. Gently mix the **3DNA UltraAmp Mix** by pipetting. Do not vortex. Protect the **3DNA UltraAmp Mix** from light.
 - 8 μ L 3DNA UltraAmp Oyster-650 (50)
 - 2 μ L Cot-1 DNA
 - 90 μ L 2X PBS + 2.5% BSA
2. Add the **3DNA UltraAmp Mix** to the wet membrane surface. Place a new 24 x 50mm glass coverslip on top of the liquid film, taking care not to trap air bubbles under the coverslip. If air bubbles are present, gently move the coverslip so that the air bubbles migrate to the edge of the coverslip.
3. Incubate for 60 minutes at room temperature (20-25°C) in the dark.

Washing the Array

1. Immerse the array in 1X PBS to float the coverslip off.
2. Fully immerse the array into a suitable container (a discarded pipette box lid works well) pre-filled with 25-50mL of 1X S&S Washing Buffer or 1X PBS + 0.02% Tween-20. Wash with vigorous agitation on an orbital shaker at room temperature for 5-10 minutes.
3. Repeat the washes twice with fresh changes of washing buffer. For a total of three washes.
4. Dry the array for 5 minutes in a 65°C oven (or equivalent). Cool the array to room temperature prior to scanning.

Signal Detection

As quickly as possible, scan the microarray as described by the scanner's manufacturer. Avoid excess multiple scans as the dyes may photobleach from exposure to the scanner light source. Scan on a suitable microarray scanner using the 635nm laser only (the nitrocellulose membrane will auto-fluoresce with the 532nm laser).

References

Nilsen, T.W., Grayzel, J., and Prensky, W. Dendritic Nucleic Acid Structures. *J. Theor. Biol.* (1997) 187: 273-284.

Stears, R.L., Getts, R.C., and Gullans, S.R. A novel, sensitive detection system for high-density microarrays using dendrimer technology. *Physiol Genomics* (2000) 3: 93-99.

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