

## Example Procedure for the Use of 3DNA<sup>®</sup> UltraAmp<sup>™</sup> Signal Amplification Reagents with Glass Microarrays

### Other Materials Required or Recommended

- Microarray reader equipped to read Oyster<sup>®</sup>-550 or Oyster<sup>®</sup>-650 fluorochromes
- Glass array: Commercial or in-house prepared from antibodies, antigens or nucleic acid
- UltraAmp Anti-Biotin and/or Anti-FITC labeled with either 50, 350, or 900 Oyster Dyes
- Cot-1 DNA, 1 $\mu$ g/ $\mu$ 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)
- 0.2X SSC buffer
- Glass coverslips
- Glass slide staining jars (or equivalent)
- Primary analyte(s) (either labeled or unlabeled)
- Optional: Biotin Labeling Kit (Pierce)
- Optional: Fluorescein Labeling Kit (Pierce)
- Optional: Biotinylated secondary antibody
- Optional: FITC-labeled secondary antibody
- Optional: DyeSaver<sup>®</sup> 2 (Genisphere cat. no. Q500500)

### 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. Make fresh 2X PBS + 2.5% BSA. Use within a few hours of preparation; alternatively, this solution may be frozen in small aliquots for future use. Add 4 $\mu$ L of Cot-1 DNA to every 100 $\mu$ L of 2X PBS + 2.5% BSA. Vortex to mix.
2. Carefully add 100 $\mu$ L of the blocking solution to the microarray. Cover the entire array area with a new glass coverslip.
3. Incubate in a humidified 37°C environment for 1-2 hours. During this incubation, prepare the primary analyte (see below).
4. Just prior to adding the next reagent, float off the array coverslip in 1X PBS + 0.02% Tween-20. This should take only a few seconds of immersion in the buffer. Blot the slide on a paper towel to remove excess liquid, and immediately apply the primary analyte (see below).

## Binding of Primary Analyte

The primary analyte (antigen, antibody, or nucleic acid) 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.
2. Add 60-100 $\mu$ L of the sample to the array, taking care not to touch the array surface. Cover the entire array area with a new glass coverslip.
3. Incubate in a humidified 37°C environment for 30-60 minutes. During this incubation, begin to pre-warm 3 containers of 1X PBS + 0.02% Tween-20 to 37°C (see below).

## Washing the Array

1. Float off the array coverslip from the previous step in 1X PBS + 0.02% Tween-20 at room temperature. This should take only a few seconds of immersion in the buffer.
2. Immediately transfer the slide into a container pre-filled with 1X PBS + 0.02% Tween-20 pre-warmed to 37°C. A slide staining container that holds the slides above a spinning stir bar is preferred; otherwise, the container should be capable of agitation during the washing procedure.
3. Wash the slide for 5-10 minutes with agitation.
4. Repeat steps 2 and 3 twice for a total of three washes.
5. 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 Detection Reagent**.  
If the primary analyte was hapten labeled, proceed to **3DNA UltraAmp Hybridization**.

## Binding of Secondary Detection Reagent

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.
2. Add 60-100 $\mu$ L of the diluted secondary detection reagent to the array, taking care not to touch the array surface. Cover the entire array area with a new glass coverslip.
3. Incubate in a humidified 37°C environment for 15-60 minutes. During this incubation, begin to pre-warm 3 containers of 1X PBS + 0.02% Tween-20 to 37°C (see below).

## Washing the Array

1. Float off the array coverslip from the previous step in 1X PBS + 0.02% Tween-20. This should take only a few seconds of immersion in the buffer.
2. Immediately transfer the slide into a container pre-filled with 1X PBS + 0.02% Tween-20 pre-warmed to 37°C. A slide staining container that holds the slides above a spinning stir bar is preferred; otherwise, the container should be capable of agitation during the washing procedure.
3. Wash the slide for 10 minutes with agitation.
4. Repeat steps 2 and 3 twice for a total of three washes. During the washes, prepare a **3DNA UltraAmp Mix** for each array (see below).
5. Proceed directly to **3DNA UltraAmp Hybridization**. Do not allow the slide to dry.

## 3DNA UltraAmp Hybridization

1. Prior to completing the wash steps, prepare a **3DNA UltraAmp Mix** for each array. Do not exceed 100 $\mu$ L as the final volume per array. Gently mix the **3DNA UltraAmp Mix** with by pipetting. Do not vortex. Protect the **3DNA UltraAmp Mix** from light.

Desired Volume:	60 $\mu$ L	80 $\mu$ L	100 $\mu$ L
3DNA UltraAmp reagent #1	8 $\mu$ L	8 $\mu$ L	8 $\mu$ L
3DNA UltraAmp reagent #2**	8 $\mu$ L	8 $\mu$ L	8 $\mu$ L
2X PBS + 2.5% BSA (half the final volume)	30 $\mu$ L	40 $\mu$ L	50 $\mu$ L
Cot-1 DNA	2 $\mu$ L	2 $\mu$ L	2 $\mu$ L
Nuclease-Free Water	12 $\mu$ L	22 $\mu$ L	32 $\mu$ L

\*\*Note: For single-color arrays, use Nuclease-Free Water in place of the second UltraAmp reagent.

2. Add the **3DNA UltraAmp Mix** directly to the wet microarray slide surface. Place a new glass coverslip of appropriate size 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 in a dark humidified 37°C environment for the appropriate time:  
UltraAmp 50 labels: 2-3 hours  
UltraAmp 350 labels: 3-4 hours  
UltraAmp 900 labels: 4-6 hours

Note: During this incubation, begin to pre-warm 3 containers of 1X PBS + 0.02% Tween-20 to 37°C (see below).

## Washing the Array

1. Float off the array coverslip in 1X PBS + 0.02% Tween-20 (room temperature). This should take only a few seconds of immersion in the buffer.

Note: If the coverslip is difficult to remove, this may be an indication of drying. To prevent this problem from recurring in future experiments, increase the total volume of the **3DNA UltraAmp Mix** by adding equal volumes of Nuclease-Free Water and 2X PBS + 2.5% BSA. In addition, ensure that the incubation chamber is properly humidified and sealed.

2. Immediately transfer the slide into a new container of 37°C 1X PBS + 0.02% Tween-20. A slide staining container that holds the slides above a spinning stir bar is preferred; otherwise, the container should be capable of agitation during the washing procedure.
3. Wash the slide for 10 minutes with vigorous agitation at 37°C.
4. Repeat steps 2 and 3 twice for a total of three washes with 1X PBS + 0.02% Tween-20.
5. Perform a final soak in 0.2X SSC for 1 minute, without agitation, at room temperature.
6. To dry the slide, immediately transfer the array to a dry 50mL centrifuge tube. Do this quickly to avoid streaky background on the slide. Orient the slide so that any label is down in the tube. Centrifuge without the tube cap for 2-3 minutes at 1000-1200 RPM to dry the slide. Avoid contact with the array surface.

**Optional:** Apply DyeSaver 2 (Genisphere cat. no. Q500500) to preserve fluorescent signal.

## 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.

## References

Nilsen, T.W., Grayzel, J., and Prenskey, 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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