# Applied Biosystems Chemiluminescence Detection Kit

Protocol

Applied Biosystems

**For Research Use Only. Not for use in diagnostic procedures.**



**TRADEMARKS:**



Part Number 4339627 Rev. D
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# Contents

# Preface

This preface contains:



## Safety

### Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word–**IMPORTANT, CAUTION, WARNING, DANGER**–implies a particular level of observation or action, as defined below:

**IMPORTANT!** – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.


– Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.


– Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.


– Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

### Chemical Hazard Warning


**CHEMICAL HAZARD**. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

### Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page vi.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

### About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

### Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose
4. To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
5. After you enter the required information, click **View/Deliver Selected Documents Now**.

### Chemical Waste Hazard


**CHEMICAL WASTE HAZARD.** Some wastes produced by the operation of the instrument or system are potentially hazardous and can cause injury, illness, or death.

### Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)

- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

### Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

**IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

### Biological Hazard Safety


**BIOHAZARD.** Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **http://bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**).

Additional information about biohazard guidelines is available at: **http://www.cdc.gov**

## How to Obtain Support

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

# Overview

## Purpose

Use the Applied Biosystems Chemiluminescence Detection Kit (PN 4342142) to:

- Hybridize digoxigenin (DIG)-labeled cRNA or cDNA targets to probes on an Applied Biosystems microarray
- Prepare the microarray for chemiluminescence (CL) detection in the Applied Biosystems 1700 Chemiluminescent Microarray Analyzer

## Kit Contents

The CL detection kit contains:

- cRNA fragmentation reagents
- Hybridization reagents and controls
- Chemiluminescence reaction reagents

## About This Protocol

This protocol provides:

- Information about kit capacity, components, and storage
- A list of required and optional equipment and materials not supplied with the kit
- Procedures for using the CL detection kit

## Process Illustration

Figure 1 illustrates the chemiluminescence detection process using the CL detection kit.

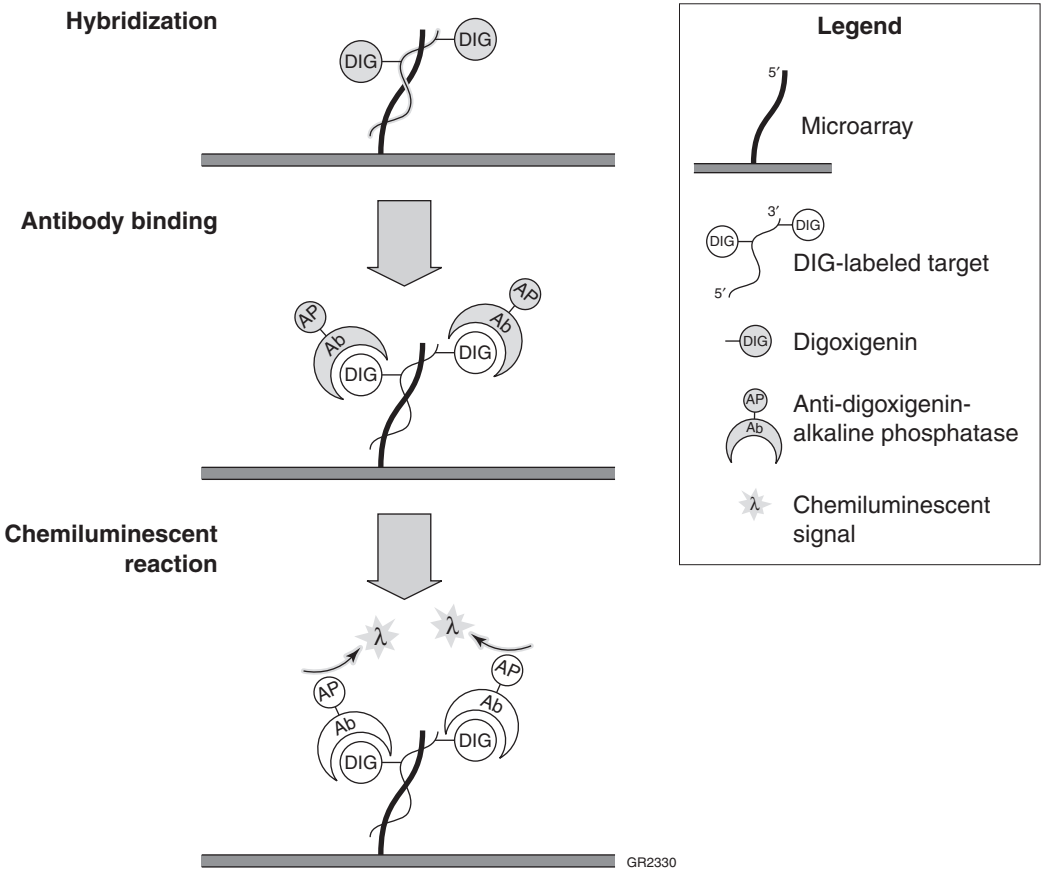



**Figure 1 Chemiluminescence detection process**

# Materials and Equipment

## Kit Capacity

The Applied Biosystems Chemiluminescence Detection Kit (PN 4342142) contains materials sufficient to perform:

- Prehybridization, hybridization, and detection of 12 microarrays
- Hybridization washes for 5 sets of microarrays (up to 4 microarrays per set)

## Components and Storage

The kit is packaged in two separate boxes.

**Table 1 Kit components**

| Component | Packaging | Quantity | Storage Temperature |
|---|---|---|---|
| Box 1 of 2 | | | |
| Blocking Reagent | 1 bottle | 24 mL | 2 to 8 °C |
| Chemiluminescence Enhancing Rinse Concentrate | 1 bottle | 60 mL | 2 to 8 °C |
| Chemiluminescence Enhancing Solution | 1 bottle | 48 mL | 2 to 8 °C |
| Chemiluminescence Substrate | 1 bottle | 42 mL | 2 to 8 °C |
| cRNA Fragmentation Buffer | 1 tube | 120 µL | 2 to 8 °C |
| cRNA Fragmentation Stop Buffer | 1 tube | 600 µL | 2 to 8 °C |
| Hybridization Buffer | 1 bottle | 6 mL | 2 to 8 °C |
| Hybridization Controls | 1 tube | 360 µL | 2 to 8 °C |
| Hybridization Denaturant | 1 tube | 1.8 mL | 2 to 8 °C |
| Transparent colored dots (5 colors) for quick reagent identification | 1 sheet | | Ambient |
| Box 2 of 2 | | | |
| Chemiluminescence Rinse Buffer Concentrate, 20× | 2 bottles | 205 mL each | Ambient |

Table 1 Kit components *(continued)*

| Component | Packaging | Quantity | Storage Temperature |
|---|---|---|---|
| Hybridization Wash Buffer Concentrate, 10× | 1 bottle | 245 mL | Ambient |
| Hybridization Wash Detergent Concentrate, 5× | 2 bottles | 245 mL each | Ambient |

## Ordering Microarrays

Visit the Applied Biosystems Web site or contact your Applied Biosystems sales representative to order available products:

**www.appliedbiosystems.com**

## Equipment and Materials Not Included

Tables 2 and 3 include equipment and materials not included with the CL detection kit. Unless otherwise noted, many of the items are available from major laboratory suppliers (MLS).

Table 2 Applied Biosystems instruments

| Instrument | Source |
|---|---|
| Applied Biosystems 1700 Chemiluminescent Microarray Analyzer | Contact your local Applied Biosystems sales representative. |
| 96-Well GeneAmp® PCR System 9700 | |

Table 3 User-supplied materials and equipment

| Material/Equipment | Source |
|---|---|
| Required materials shipped with the Applied Biosystems 1700 Chemiluminescent Microarray Analyzer | |
| Applied Biosystems 1700 Wash Tray (12 pack) | Applied Biosystems PN 4352927 |
| Applied Biosystems 1700 Oven Clip Kit (12 clips and 24 screws) | Applied Biosystems PN 4347124 |
| Required materials and equipment | |
| Applied Biosystems Human Genome Survey Microarray (4 microarrays) | Applied Biosystems PN 4337467 |

**Table 3 User-supplied materials and equipment *(continued)***

| Material/Equipment | Source |
|---|---|
| Applied Biosystems Human Genome Survey Microarray (12 microarrays) | Applied Biosystems PN 4337468 |
| Anti-Digoxigenin-AP, Fab fragments: 150 U (200 µL) | Roche Applied Science PN 1093274 |
| Graduated cylinders: 0.5-L, 1-L, 2-L | MLS |
| Hybridization oven:<br>• VWR Signature Benchtop Shaking Incubator, Model 1575<br>• Infors HT Minitron Incubator Shaker<br>For more information, see "Recommended Hybridization Ovens" on page 7. | • VWR Scientific<br>• Shell Labs Distribution<br>• Infors HT |
| Lint-free tissue | MLS |
| Microcentrifuge | MLS |
| Microcentrifuge tubes, nuclease-free: 1.5-mL | MLS |
| VWR Rocking Platform Shaker, Single-Tier Platform:<br>• 115V, 60Hz (North America)<br>• 220V, 60Hz (Europe and Japan) | VWR Scientific<br>• PN 40000-300<br>• PN 40000-302 |
| Multi-tiered Rocker:<br>• 150V/60Hz<br>• 220V/60Hz | Stovall Hi/Lo Profile Rocker:<br>• ROCAA115S<br>• ROCAA220S |
| Pipette tips, nuclease-free: 1- to 20-µL range, 20- to 200-µL range, 100- to 1000-µL range | MLS |
| Pipettors: 1- to 20-µL range, 20- to 200-µL range, 100- to 1000-µL range | MLS |
| Serological pipets, plastic, sterile, nuclease-free | MLS |
| Vortexer | MLS |
| Optional materials and equipment | |

Table 3 User-supplied materials and equipment *(continued)*

| Material/Equipment | Source |
|---|---|
| Bulkpack MicroAmp® Reaction Tubes With Caps (10,000 tubes) | Applied Biosystems PN N801-1540 |
| MicroAmp® Autoclaved Reaction Tubes With Caps (1,000 tubes) | Applied Biosystems PN N801-0612 |
| MicroAmp® Colored Reaction Tubes With Caps (1,000 tubes) | Applied Biosystems PN N801-0840 |
| MicroAmp® Reaction Tubes With Caps (1,000 tubes) | Applied Biosystems PN N801-0540 |

## Related Documents

Table 4 Applied Biosystems documents

| Document | Part Number |
|---|---|
| *Applied Biosystems 1700 Chemiluminescent Microarray Analyzer Chemistry Guide* | 4338853 |
| *Applied Biosystems 1700 Chemiluminescent Microarray Analyzer Site Preparation Guide* | 4338850 |
| *Applied Biosystems 1700 Chemiluminescent Microarray Analyzer User Guide* | 4338852 |
| *Applied Biosystems Chemiluminescence Detection Kit Quick Reference Card* | 4346875 |
| *Applied Biosystems Chemiluminescent RT Labeling Kit Protocol* | 4339628 |
| *Applied Biosystems Chemiluminescent RT Labeling Kit Quick Reference Card* | 4346876 |
| *Applied Biosystems Chemiluminescent RT-IVT Labeling Kit Protocol* | 4339629 |
| *Applied Biosystems Chemiluminescent RT-IVT Labeling Kit Quick Reference Card* | 4346877 |

## Recommended Hybridization Ovens

### Minimum Specifications

- **Load Capacity**: > 6 kg
- **Deck Space**: 43.8 cm × 43.8 cm
- **Bolt Hole**: 58.34 mm (center-to-center)
- **Rotation**: 100 rpm
- **Temperature**: 55 ± 1 °C

### Ovens Tested by Applied Biosystems

Two hybridization ovens were tested to meet Applied Biosystems performance specifications for the CL detection kit:

- VWR Signature Benchtop Shaking Incubator, Model 1575
- Infors HT Minitron Incubator Shaker

### VWR Signature Benchtop Shaking Incubator Suppliers

**VWR Scientific (United States)**
Ordering and technical support: 1.800.932.5000
Web and catalog support: 1.888.320.4357
Web address: http://vwrsp.com

PN: 35962-091 (Platform PN 550625 Rev. A)

**VWR International (Europe)**
Haasrode Research Zone 3
Geldenaaksebaan 464
B-3001 Leuven, Belgium
Telephone: +32.15.385.167
Fax: +32.16.385.393
Email: info@be.vwr.com
Web address: http://be.vwr.com/app/Home

PN: 35962-092 (Platform PN 550625 Rev. A), specify cord

**Shell Labs Distribution (United States, Europe, and Japan)**
Oriental Giken Inc.
NC Takebashi Bldg.
2-11-3 Kanda Nishiki Cho
Chiyoda-Ku, Tokyo 101-0054 Japan
Telephone: +03.3233.0821
Fax: +03.3233.0825
E-mail: nomura@orientalgiken.co.jp
Web address: http://www.shellab.com

- PN (United States and Japan): Sheldon Model #1575 (Platform PN 550625 Rev. A)
- PN (Europe): Sheldon Model #1575-2 (Platform PN 550625 Rev. A), specify cord

### Infors HT Minitron Incubator Shaker Suppliers

**Infors HT (Worldwide)**
Web address: http://www.infors-ht.com

# Chemiluminescence Detection Procedures

## Procedure Overview

CL detection involves the following procedures:

1. Setting up the hybridization oven (page 10)
2. Prehybridizing microarrays (page 10)
3. Fragmenting cRNA (page 13)
4. Hybridizing samples to microarrays (page 14)
5. Preparing wash buffers (page 17)
6. Performing hybridization washes (page 20)
7. Performing antibody binding (page 23)
8. Performing antibody washes (page 24)
9. Performing the CL reaction (page 26)
10. Performing CL detection (page 28)

## Procedure Guidelines

For optimal performance:

- If you used the RT labeling kit and used up to 2 µg mRNA input or up to 40 µg total RNA input, use the entire DIG-labeled cDNA product (90 µL) on one microarray.
- If you used the RT-IVT labeling kit, use 10 µg DIG-labeled cRNA product.
- Use nuclease-free pipette tips and reagents.
- Use new, powder-free gloves for each procedure.
- Observe standard laboratory practices to prevent biological contamination during the preparation, storage, and use of reagents and equipment for this protocol. External RNase contamination may cause a significant loss of performance.

## Microarray Handling Precautions

The microarrays are fragile.When handling and positioning them:

- Handle the microarray by the edges.
- Do not touch the microarray surface.
- Do not allow the microarray to dry out.
- Avoid disturbing the gasket

## Selecting a Thermal Cycler

For each step that requires a thermal cycler, use the GeneAmp PCR System 9700. The heated lid minimizes evaporation and condensation in the tube cap:

**IMPORTANT!** Because of differences in ramp rates and thermal accuracy, you may need to adjust the settings if you choose to use other thermal cyclers.

## Setting Up the Hybridization Oven

The oven may take up to 3 hours to heat up and equilibrate to 55 °C.

**To set up the hybridization oven:**

| | |
|---|---|
| 1. | Power on the hybridization oven. |
| 2. | Set the temperature to 55 °C. |

## Prehybridizing Microarrays

**To prehybridize microarrays:**

| | |
|---|---|
| 1. | Verify that the oven temperature is 55 °C. |
| 2. | Warm reagents at 37 °C for 30 minutes, then vortex to dissolve any precipitates:<br>• Hybridization Buffer<br>• Hybridization Denaturant<br>• Blocking Reagent |
| 3. | Equilibrate microarrays to room temperature. |

**To prehybridize microarrays:** ***(continued)***

4. Prepare prehybridization mixture in a nuclease-free tube and vortex to mix:

| Component | Volume (µL) / Microarray |
|---|---|
| Nuclease-free water | 150 |
| Hybridization Buffer | 330 |
| Hybridization Denaturant | 100 |
| Blocking Reagent | 420 |
| **Total/Microarray** | 1000 |

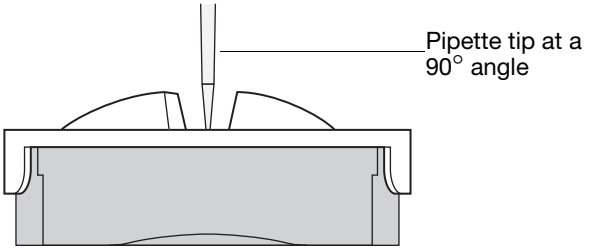
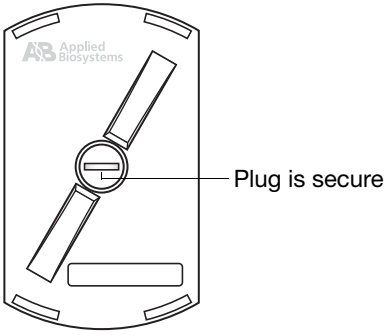
**Note:** When calculating volumes for multiple microarrays, add 10% to provide for the loss that occurs during reagent transfers.

**WARNING** **CHEMICAL HAZARD**. **Hybridization Buffer** causes eye irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**WARNING** **CHEMICAL HAZARD**. **Hybridization Denaturant** causes eye, skin, and respiratory tract irritation. It is harmful if swallowed. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**WARNING** **CHEMICAL HAZARD**. **Blocking Reagent** causes eye irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**To prehybridize microarrays:** ***(continued)***

| | |
|---|---|
| 5. | Transfer prehybridization mixture into each Applied Biosystems microarray cartridge (repeat for each cartridge):<br>a. Remove the cartridge from the foil wrapping.<br>b. Remove the plug and pipette the prehybridization mixture (1 mL) into the port with the pipette tip at a 90° angle.<br>  <br><br>c. Dry the outer surface of the port with lint-free tissue and seal the port with the plug. Turn the plug 90 degrees to secure it.<br>  <br><br>d. Gently rock and tilt the cartridge to ensure complete wetting of the microarray surface.<br>**IMPORTANT!** Do not shake the cartridge.<br>e. Rock all cartridges in a vertical orientation on the rocking platform for 5 minutes at room temperature. |
| 6. | Place all cartridges in the oven, making sure that each cartridge is level and held securely by the clamps, then close the oven door.<br>**Note:** You may stack two cartridges in one clip. |

**To prehybridize microarrays: *(continued)***

| | |
|---|---|
| 7. | Incubate the cartridges in the hybridization oven:<br>• Temperature: 55 °C<br>• Agitation: 100 rpm<br>• Time: 1 hour |

## Fragmenting cRNA

During prehybridization, fragment the cRNA if you used the Applied Biosystems Chemiluminescent RT-IVT Labeling Kit and have DIG-labeled cRNA targets.

If you used the Applied Biosystems Chemiluminescent RT Labeling Kit and have DIG-labeled cDNA, proceed with "Hybridizing Samples to Microarrays" on page 14 after prehybridization.

**To fragment cRNA:**

| | |
|---|---|
| 1. | Allow reagents to equilibrate to room temperature for 1 hour, then vortex and centrifuge briefly before use:<br>• cRNA Fragmentation Buffer<br>• cRNA Fragmentation Stop Buffer |
| 2. | Combine the following components in a MicroAmp reaction tube on ice, then mix by pipetting: |

| Component | Volume (µL) |
|---|---|
| cRNA Fragmentation Buffer | 10.0 |
| 10 µg DIG-labeled cRNA and nuclease-free water | 90.0 |
| **Total** | 100.0 |

**WARNING** **CHEMICAL HAZARD. cRNA Fragmentation Buffer** causes eye and skin irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To fragment cRNA: *(continued)*

| | |
|---|---|
| 3. | Heat the tube in a thermal cycler:<br><br>**Temperature** \| **Time** \| **Reaction Volume**<br>60 °C \| 30 minutes \| 100 µL |
| 4. | Neutralize the reaction:<br>a. Remove the tube from the thermal cycler, then centrifuge the tube briefly.<br>b. Add 50 µL of cRNA Fragmentation Stop Buffer, then mix by pipetting.<br>c. Place the tube on ice.<br><br>**WARNING** **CHEMICAL HAZARD. cRNA Fragmentation Stop Buffer** causes eye irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. |

## Hybridizing Samples to Microarrays

To hybridize samples to microarrays:

| | |
|---|---|
| 1. | Vortex Hybridization Controls, then centrifuge the tube briefly. |
| 2. | If you used the Applied Biosystems Chemiluminescent RT Labeling Kit, add nuclease-free water to the cDNA targets to bring the volume to 150 µL.<br><br>**Note:** If you used up to 2 µg mRNA input or up to 40 µg total RNA input, use the entire DIG-labeled cDNA product (90 µL) on one microarray. |

**To hybridize samples to microarrays:** ***(continued)***

3. For each microarray, prepare hybridization mixture in a nuclease-free microcentrifuge tube:

| Component | Volume (µL) / Microarray |
|---|---|
| Nuclease-free water | 100 |
| Hybridization Buffer | 170 |
| Hybridization Controls | 30 |
| cDNA and nuclease-free water or fragmented cRNA targets | 150 |
| Hybridization Denaturant | 50 |
| **Total/Microarray** | 500 |


**CHEMICAL HAZARD**. **Hybridization Buffer** causes eye irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.


**CHEMICAL HAZARD**. **Hybridization Denaturant** causes eye, skin, and respiratory tract irritation. It is harmful if swallowed. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

4. Vortex the hybridization mixture, then centrifuge the tube briefly.

5. Take to the hybridization oven:
   - Tube(s) with hybridization mixture
   - 1000-µL pipettor
   - 1000-µL pipette tips
   - Lint-free tissues

**To hybridize samples to microarrays: *(continued)***

| | |
|---|---|
| 6. | *Quickly* transfer hybridization mixture into each Applied Biosystems microarray cartridge (repeat for each cartridge):<br><br>**IMPORTANT!** Add hybridization mixture quickly.<br><br>a. Remove one cartridge from the oven and close the door.<br>b. Remove the plug and pipette hybridization mixture (500 µL) into the port.<br>c. Dry the port with lint-free tissue and seal it with the plug. Turn the plug 90 degrees to secure it.<br>d. Return the cartridge to the oven, making sure that the cartridge is level and held securely by the clamps. |
| 7. | Incubate the cartridges in the hybridization oven:<br>• Temperature: 55 °C<br>• Agitation: 100 rpm<br>• Time: 16 hours<br><br>**IMPORTANT!** Do not vary the incubation time. Variations may affect reproducibility. |

## Wash Buffer Preparation Guidelines

To avoid nuclease contamination in wash buffers:

- Make wash buffers the same day that you plan to use them.
- Warm all wash reagents to 22 °C before use.
- Wear new, clean gloves at all times.
- Use nuclease-free graduated cylinders and serological pipettes.
- If using cRNA targets, take all precautions to avoid RNase contamination.

## Preparing Wash Buffers and Reagents

Volumes provided are for one set of washes with one to four microarrays in one AB 1700 wash tray.

**To prepare wash buffers and reagents:**

1. Allow the following reagents to equilibrate to 22 °C before use:
   - Hybridization Wash Buffer Concentrate
   - Hybridization Wash Detergent Concentrate
   - Chemiluminescence Rinse Buffer Concentrate
   - Chemiluminescence Enhancing Rinse Concentrate
   - Nuclease-free deionized water
2. Heat the following reagents to 37 °C for 30 minutes, then mix well. Allow to equilibrate to room temperature before use:
   - Chemiluminescence Enhancing Solution
   - Blocking Reagent
3. Prepare hybridization wash buffer 1:

| Component | Volume (mL) |
|---|---|
| Hybridization Wash Buffer Concentrate | 30 |
| Hybridization Wash Detergent Concentrate | 60 |
| Nuclease-free deionized water | 210 |
| **Total** | 300 |


**CHEMICAL HAZARD.** **Hybridization Wash Buffer Concentrate** causes eye irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**To prepare wash buffers and reagents:** ***(continued)***

4. Prepare hybridization wash buffer 2:

| Component | Volume (mL) |
|---|---|
| Hybridization Wash Buffer Concentrate | 1.5 |
| Nuclease-free deionized water | 298.5 |
| **Total** | 300.0 |

**WARNING** **CHEMICAL HAZARD.**
**Hybridization Wash Buffer Concentrate** causes eye irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

5. Prepare CL rinse buffer:

| Component | Volume (mL) |
|---|---|
| Chemiluminescence Rinse Buffer Concentrate | 75 |
| Nuclease-free deionized water | 1425 |
| **Total** | 1500 |

**WARNING** **CHEMICAL HAZARD.**
**Chemiluminescence Rinse Buffer Concentrate** causes eye irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**To prepare wash buffers and reagents: *(continued)***

6. Prepare CL enhancing rinse buffer:

| Component | Volume (mL) |
|---|---|
| Chemiluminescence Enhancing Rinse Concentrate | 15 |
| Nuclease-free deionized water | 585 |
| **Total** | 600 |

## Decontaminating Wash Trays

Applied Biosystems recommends that you decontaminate the wash trays to remove and avoid RNase contamination. Removing and avoiding RNase contamination is especially important if you are using DIG-labeled cRNA targets. Below is one suggested procedure for decontaminating wash trays or use a commercially available reagent.

**To decontaminate wash trays:**

1. Wash the wash tray with detergent; rinse well with nuclease-free deionized water.
2. Rinse with 95% ethanol.

   **WARNING** **CHEMICAL HAZARD. Ethanol** is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation and may cause central nervous system depression and liver damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3. Spray the wash tray thoroughly with 3% peroxide and wait 10 minutes.
4. Rinse the wash tray with RNase-free water.

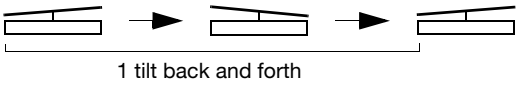
## Rocking Platform Shaker Tilt Settings

Verify the tilt settings of the recommended VWR rocking platform shaker before performing hybridization washes, antibody binding, antibody washes, and the CL reaction:

- Tilt angle: 10°

**Note:** The angle can be verified by ensuring that the height of the edge of the platform divided by the platform length is equal to cos(10°).

- Tilt speed: 30 tilts back and forth per minute




**Note:** Do not rely on the tachometer of the rocking platform shaker. Verify the speed manually.

**IMPORTANT!** Do not allow the microarrays to dry out. After completing a step, *immediately* proceed to the next step. Perform all steps in AB wash trays, one tray at a time, with up to 4 arrays per tray. Do not shake loose the secured microarray. Do not touch the microarray surface.

## Performing Hybridization Washes

Perform the hybridization washes at room temperature (19 to 30 °C). Use the tilt settings for the rocking platform shaker specified above.

**To perform hybridization washes:**

| | |
|---|---|
| 1. | Add 300 mLs of hybridization wash buffer 1 to a clean wash tray. |

**To perform hybridization washes: *(continued)***

| | |
|---|---|
| 2. | *Quickly* transfer each microarray, one at a time, from the oven to the wash tray (repeat for each microarray):<br><br>**IMPORTANT!** Do not allow cartridges to remain stationary for longer than 1 minute.<br><br>a. Open the hybridization oven door and wait for the agitation to stop. Remove one cartridge from the oven and close the door to restart agitation.<br><br>b. Open the cartridge and take the microarray out. Make sure that some hybridization volume (>0.75 mL) remains.<br><br>c. Decant the liquid, then shake and tap the microarray gently to remove excess liquid.<br><br>d. Grasp microarray by the edge of the glass slide and insert into one of four locking chambers in the wash tray. Ensure microarray is properly inserted.<br><br>**Note:** The microarray should be loose in the individual chamber, but remain in the tray when tilted.<br><br>**IMPORTANT!** Do not touch the microarray surface. |
| 3. | Perform hybridization wash 1:<br><br>a. Place the wash tray on the rocker with the arrays vertically arranged in the wash tray. Agitate on the rocking platform for 5 minutes.<br><br>b. Decant the buffer by tilting the wash tray.<br><br>**IMPORTANT!** Do not shake loose the secured microarray.<br><br>**IMPORTANT!** Proceed to the next step immediately to prevent the microarray from drying out. |

**To perform hybridization washes:** ***(continued)***

| | |
|---|---|
| 4. | Perform hybridization wash 2:<br>a. Add 300 mLs of hybridization wash buffer 2 to the wash tray, then make sure that all microarrays are submerged in buffer.<br>b. Agitate on the rocking platform for 5 minutes.<br>c. Decant the buffer by tilting the wash tray.<br>**IMPORTANT!** Do not shake loose the secured microarray.<br>**IMPORTANT!** Proceed to the next step immediately to prevent the microarray from drying out. |
| 5. | Perform CL rinse 1:<br>a. Add 300 mLs of CL rinse buffer to the wash tray, then make sure that all microarrays are submerged in buffer.<br>b. Agitate on the rocking platform for 5 minutes.<br>c. Decant the buffer by tilting the wash tray.<br>**IMPORTANT!** Do not shake loose the secured microarray.<br>**IMPORTANT!** Proceed to the next step immediately to prevent the microarray from drying out. |
| 6. | Perform CL rinse 2:<br>a. Add 300 mLs of CL rinse buffer to the wash tray, then make sure that all microarrays are submerged in buffer.<br>b. Agitate on the rocking platform for 5 minutes.<br>c. Cover the wash tray, remove it from the rocking platform shaker, then place it on the bench top.<br>**Note:** You may leave the microarrays in CL rinse buffer at room temperature for up to 1 hour. |

## Performing Antibody Binding

Use the rocking platform shaker tilt settings specified on page 20.

**To perform antibody binding:**

1. Combine components for the CL blocking buffer/antibody mixture in a nuclease-free tube and mix well by inversion. Do not vortex.

| Component | Volume/ Microarray |
|---|---|
| Nuclease-free water | 2.8 mL |
| Chemiluminescence Rinse Buffer Concentrate | 0.2 mL |
| Blocking Reagent | 1.0 mL |
| Anti-digoxigenin-AP | 15 µL |
| **Total/Microarray** | 4.015 mL |

**Note:** When calculating volumes for multiple microarrays, add 10% for the loss that occurs during reagent transfers.

**Note:** Pipette the anti-digoxigenin-AP from the center of the solution to avoid any aggregates at the bottom.

**WARNING CHEMICAL HAZARD.** **Chemiluminescence Rinse Buffer Concentrate** causes eye irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**WARNING CHEMICAL HAZARD. Blocking Reagent** causes eye irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

2. Decant the CL Rinse Buffer from the wash tray, leaving the microarrays secured.

**To perform antibody binding: *(continued)***

| | |
|---|---|
| 3. | *Immediately* add 4 mL of CL blocking buffer/antibody mixture to each microarray.<br>**IMPORTANT!** Complete this step for all microarrays within the tray in 2 minutes. Do not let the microarray dry out. |
| 4. | Agitate on the rocking platform for 20 minutes at room temperature. |

## Performing Antibody Washes

Use the rocking platform shaker tilt settings specified on page 20.

**To perform antibody washes:**

| | |
|---|---|
| 1. | Perform CL rinse 1:<br>a. Decant the CL blocking buffer/antibody mixture from the microarrays by tilting the wash tray.<br>**IMPORTANT!** Do not shake loose the secured microarrays.<br>b. Add 300 mL of CL rinse buffer to the wash tray.<br>c. Make sure that all microarrays are submerged in buffer.<br>d. Agitate on the rocking platform for 10 minutes.<br>e. Decant the CL rinse buffer from the microarrays by tilting the wash tray.<br>**IMPORTANT!** Do not shake loose the secured microarrays.<br>**IMPORTANT!** Proceed to the next step immediately to prevent the microarray from drying out. |
| 2. | Perform CL rinse 2:<br>a. Add 300 mL of CL rinse buffer to the wash tray, then make sure that all microarrays are submerged in buffer.<br>b. Agitate on the rocking platform for 10 minutes.<br>c. Decant the CL blocking buffer/antibody mixture from the microarrays by tilting the wash tray.<br>**IMPORTANT!** Do not shake loose the secured microarrays.<br>**IMPORTANT!** Proceed to the next step immediately to prevent the microarray from drying out. |

**To perform antibody washes:** ***(continued)***

| | |
|---|---|
| 3. | Perform CL rinse 3:<br>a. Add 300 mL of CL rinse buffer to the wash tray, then make sure that all microarrays are submerged in buffer.<br>b. Agitate on the rocking platform for 10 minutes.<br>c. Stop the agitation of the rocking platform . |

## Performing the CL Reaction

Use the rocking platform tilt settings specified on page 20.

**To perform the CL reaction:**

| | |
|---|---|
| 1. | Power on the 1700 instrument at least 10 minutes before using it. |
| 2. | Perform a CL enhancing rinse:<br>a. Decant the CL Rinse buffer from the microarrays by tilting the wash tray.<br>**IMPORTANT!** Do not shake loose the secured microarrays.<br>b. *Immediately* add 300 mL of CL enhancing rinse buffer to the wash tray.<br>c. Agitate the tray on the rocking platform for 10 minutes.<br>d. Stop the agitation of the rocking platform . |
| 3. | Perform the enhancing step:<br>a. Decant the CL enhancing rinse buffer from the microarrays by tilting the wash tray.<br>**IMPORTANT!** Do not shake loose the secured microarrays.<br>b. *Immediately* add 4 mL of Chemiluminescence Enhancing Solution to each microarray.<br>c. Cover the tray, then agitate the tray on the rocking platform for 20 minutes.<br>**WARNING** **CHEMICAL HAZARD**. **Chemiluminescence Enhancing Solution** causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. |

**To perform the CL reaction:** ***(continued)***

| | |
|---|---|
| 4. | Perform a CL enhancing rinse:<br>a. Decant the CL Enhancing Solution from the microarrays by tilting the wash tray.<br>**IMPORTANT!** Do not shake loose the secured microarrays.<br>b. Add 300 mL CL enhancing rinse buffer to the wash tray.<br>c. Agitate the wash tray on the rocking platform for 5 minutes.<br>d. Remove the wash tray from the rocking platform .<br>**Note:** Arrays are stable in CL enhancing rinse buffer for up to 3 hours. |
| 5. | **IMPORTANT!** The chemiluminescent reaction is time-dependent. Perform this procedure with only one microarray at a time.<br>Add substrate:<br>a. Remove one microarray from the wash tray. Decant CL enhancing rinse buffer, then shake and tap the microarray gently.<br>b. Wipe the bottom of the microarray with lint-free tissue.<br>c. Add 3.5 mL of Chemiluminescence Substrate to the microarray.<br>**IMPORTANT!** The chemiluminescent reaction is time-dependent. After you perform this step, proceed to the next procedure immediately.<br>**WARNING** **CHEMICAL HAZARD.** **Chemiluminescence Substrate** causes eye and respiratory tract irritation. Exposure may cause central nervous system depression. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. |

## Performing CL Detection

For more instructions about how to use the instrument or software, see the *Applied Biosystems 1700 Chemiluminescent Microarray Analyzer User Guide* (PN 4338852)

**To perform CL detection:**

| | |
|---|---|
| 1. | Power on the 1700 instrument. |
| 2. | Load the microarray into the instrument:<br>a. Dry any excess liquid from the bottom of the microarray with lint-free tissue.<br>b. Open the instrument door.<br>c. Orient the microarray so that the end bar code is at the top and the side bar code is on the right, then place the microarray on the heated stage of the instrument.<br>d. Lock the bar, then close the door. |
| 3. | On the computer, select **Start > Programs > Applied Biosystems > AB1700 Software** to start the software. |
| 4. | Enter the Login Name and Password assigned to you by the administrator. |
| 5. | Select the cartridge settings:<br>• Microarray Type<br>• Chemistry Method<br>• Reader Method<br>• Analysis Method<br>• Folder<br>• Sample Name/ID |
| 6. | Click **Start** and wait for the instrument to obtain the images. |
| 7. | Analyze the data, using the following documents:<br>• *Applied Biosystems 1700 Chemiluminescent Microarray Analyzer User Guide* (PN 4338852)<br>• *Applied Biosystems 1700 Chemiluminescent Microarray Analyzer Chemistry Guide* (PN 4338853) |

**Headquarters**
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

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