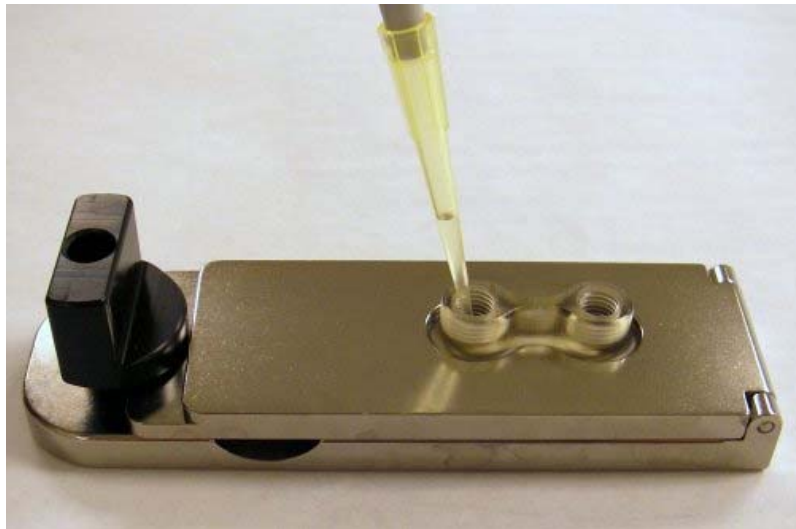


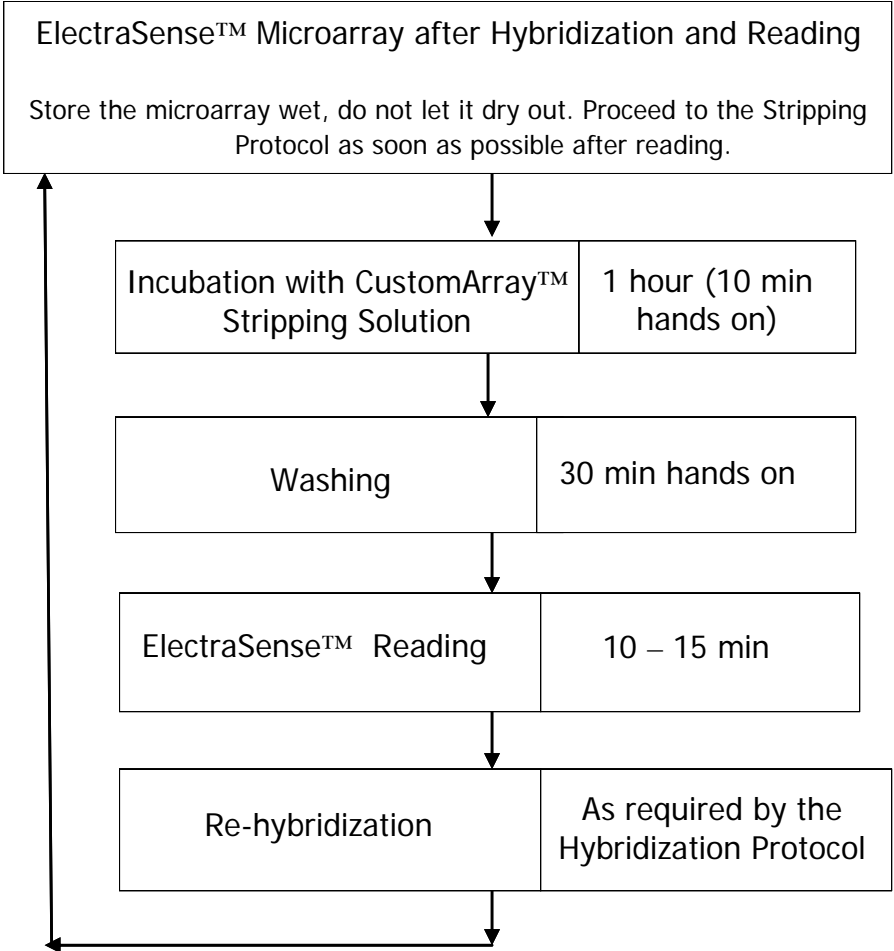
# COMBIMATRIX

## Stripping and Preparation of ElectraSense™ 12K Microarrays for Re-hybridization (PTL003)

ElectraSense™ Stripping Kit for 12K, Product Number 610029



# Stripping and Preparation of ElectraSense™ 12K Microarrays for Re-hybridization Protocol Workflow



# Stripping and Preparation of ElectraSense™ 12K Microarrays for Re-hybridization

## Table of Contents

Table of Contents .....	ii
Introduction .....	1
Materials and Equipment Provided with ElectraSense™ Stripping Kit.....	2
Materials and Equipment Required (not provided).....	2
ElectraSense™ 12K Assembly with Stripping Cap, Gasket and Clamp .....	3
Stripping of ElectraSense™ Microarray.....	5
Washing of ElectraSense™ Microarray after Stripping.....	6
ElectraSense™ Reading after Stripping .....	6
Preparation of Stripped ElectraSense™ Microarray for Re-hybridization.....	7
Storage of Stripped ElectraSense™ Microarray.....	7

# Stripping and Preparation of ElectraSense™ 12K Microarrays for Re-hybridization

## Introduction

This manual describes how to strip labeled RNA and/or DNA targets from CombiMatrix ElectraSense™ 12K microarrays using the ElectraSense™ Stripping Kit (Product Number 610029), and how to prepare the stripped microarrays for re-hybridization with new labeled targets. The stripping procedure is based on denaturation of DNA:DNA or DNA:RNA hybrids, resulting in labeled targets being removed from the oligonucleotide probes and subsequently washed off the microarray. Stripping and re-hybridization may be repeated three times; therefore, each microarray can be used up to a maximum of four times. CombiMatrix does not recommend increasing the number of re-uses.

The stripping protocol is recommended for the ElectraSense™ microarrays that have standard oligonucleotide probe length of 40-mer or less. Probes that are longer than 40-mer will not be stripped completely. Effective stripping also depends on microarray handling during hybridization. The following issues may result in incomplete stripping:

- ❑ Drying of the semiconductor surface while targets are hybridized to microarray probes will invariably preclude stripping of the microarray. Once the hybridized nucleic acids are dried, they cannot be removed.
- ❑ Nucleic acid targets of large size tend to precipitate in the porous reaction layer covering the microarrays semiconductor and will not strip easily. Low molecular weight target material must be used for stripping to be effective. To achieve optimum hybridization and stripping results, target samples need to be fragmented. The fragment size should be in the range of 50 to 200 nucleotide for RNA, and from 50 to 500 nucleotide for DNA, respectively.
- ❑ Microarray overloading with targets during hybridization. CombiMatrix recommends to use no more than 5 microgram for a 12K microarray. In some cases, saturated (i.e. overloaded) spots cannot be completely stripped, and then the concentration of target material should be reduced for efficient stripping.

The quality of stripping must be determined prior to re-hybridization. This is achieved by reading of the stripped microarray with the ElectraSense™ Reader instrument. The re-hybridization of the stripped microarray may be performed in the same way as the initial hybridization. For details please refer to the corresponding ElectraSense™ Hybridization Protocol for 12K microarrays. New ElectraSense™ Hybridization Caps and Clips for re-uses are provided with the ElectraSense™ Stripping Kit.

## Materials and Equipment Provided with ElectraSense™ Stripping Kit

One kit is intended for 25 uses.

ElectraSense™ Stripping Kit for 12K	Quantity
Stripping Cap (multiple use), 0.5 ml volume	1
Disposable Gaskets (O-rings) for Stripping Cap	25
Screw Plugs for Stripping Cap	2
CustomArray™ Stripping Solution	1 bottle of 25 ml
ElectraSense™ Hybridization Caps (for Re-hybridization)	25
Clips for Hybridization Caps (for Re-hybridization)	50

---

**IMPORTANT! The CustomArray™ Stripping Solution contains ethanolamine, which is highly corrosive and can cause severe burns. The solution must be handled under a fume hood at all times. Wear protective clothing including gloves, lab coat, and goggles at all times. Follow all instructions for handling and disposal as indicated in the Material Safety Data Sheet (MSDS) provided.**

---

## Materials and Equipment Required (not provided)

- ❑ CombiMatrix CustomArray™ Stripping Clamp for 12K (multiple use up to 100 times), Product Number 610010

---

NOTE: The CustomArray™ Stripping Clamp may be used up to 100 times. Some staining may occur on the surface of Clamp from spilled Stripping Solution, however, this will not affect the Clamp performance. The Stripping Cap and Screw Plugs supplied with the kit can be used for 25 stripping reactions.

---

- ❑ 65°C Incubator
- ❑ 37°C Incubator
- ❑ CombiMatrix ElectraSense™ Reader
- ❑ Wet Foam Swabs (provided with the ElectraSense™ Reader, if necessary, additional swabs can be obtained from Tansen, Item # CLTX- 815, [http://www.cleantex.org/Swabs\\_Foam\\_wet.html](http://www.cleantex.org/Swabs_Foam_wet.html) or [sale@tansen.com](mailto:sale@tansen.com))
- ❑ CombiMatrix ElectraSense™ Detection Kit, Product Number 610027
- ❑ Nuclease-free water
- ❑ 95% ethanol
- ❑ 10X PBS buffer (1.37M Sodium Chloride, 0.027M Potassium Chloride, 0.08M Sodium Phosphate dibasic, 0.02M Sodium Phosphate monobasic, pH 7.4; Ambion Cat.# 9625) diluted to 1X PBS
- ❑ Labeled target samples for re-hybridization
- ❑ Other materials required for ElectraSense™ microarray hybridization as listed in the CombiMatrix Protocols
- ❑ Micropipettors, tips, sterile plastic ware
- ❑ Powder-free gloves, Nitrile or Chloroprene (not Latex)
- ❑ Personal protection wear: lab coat and protective goggles

# ElectraSense™ 12K Assembly with Stripping Cap, Gasket and Clamp

---

**IMPORTANT! The disposable gaskets (O-rings) for the Stripping Cap supplied with the kit are intended for single use only. Re-use of gaskets may cause leakage of the Stripping Solution from the Stripping Cap. Dispose of the used gasket upon completion of this protocol.**

---

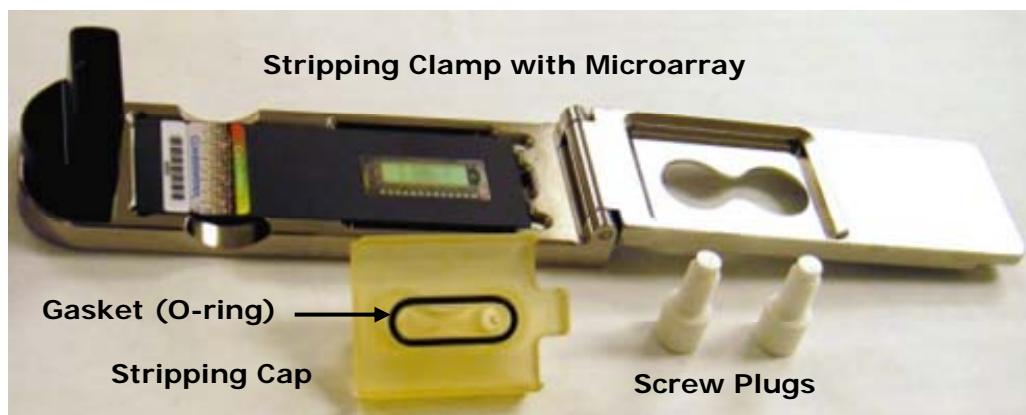
1. Wash the Stripping Cap with ethanol and dry carefully with a paper towel/tissue. Place a new gasket into the O-ring groove on the Stripping Cap (see Figure 1).
2. Open the Stripping Clamp by turning the knob counter-clockwise.
3. Remove a hybridized ElectraSense™ microarray from the ElectraSense™ Reader.
4. Pipet the TMB Solution from the hybridization chamber.
5. Wash the hybridization chamber twice with nuclease-free water, pipet water from the chamber.
6. Immediately remove the clips and lift the hybridization cap off the microarray surface.
7. The contact pads on the ElectraSense™ microarray surface need to stay dry to prevent corrosion. Immediately wipe the contact pads, the area surrounding the semiconductor array, and the back of the slide with a paper towel/tissue.

---

**IMPORTANT! Do not let the semiconductor microarray surface to dry, immediately proceed to the next step.**

---

8. Place the microarray into the Stripping Clamp with the array semiconductor side-up, and the microarray label close to the knob (see Figure 1).



**Figure 1.** Placement of a microarray in the CustomArray™ Stripping Clamp.

9. Position the Stripping Cap with the gasket-side down over the microarray, so that it covers the semiconductor array area. The arm of the Stripping Cap should fit into the stop in the stripping clamp (see Figure 2).



**Figure 2.** Position of the Stripping Cap over the microarray in the CustomArray™ Stripping Clamp.

10. Close the CustomArray™ Stripping Clamp and turn the knob clockwise to apply pressure and secure the Stripping Cap. To fill up the stripping chamber, place the Stripping Clamp horizontally and use the solution portals as shown in Figure 3.



**Figure 3.** Pipetting of solutions into the assembled CustomArray™ Stripping Clamp.

---

**IMPORTANT! Immediately proceed to stripping. Do not allow the array to become dry at any step in the protocol. Proceed rapidly when changing solutions during washing steps. Do not leave the hybridization chamber empty for any significant length of time.**

---

## Stripping of ElectraSense™ Microarray

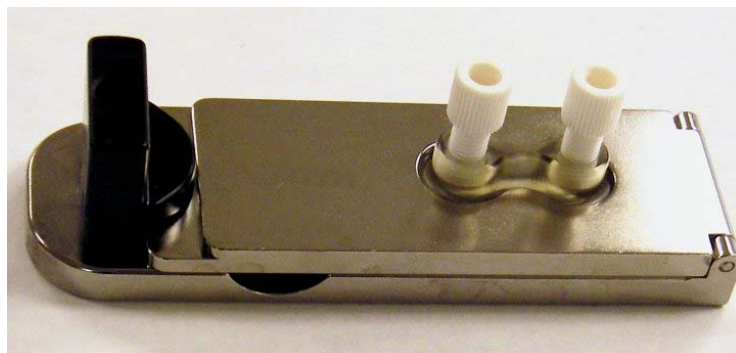
1. Transfer the assembled CustomArray™ Stripping Clamp into a fume hood. Wear gloves, a lab coat and protective goggles.

---

**IMPORTANT!** The CustomArray™ Stripping Solution contains ethanolamine, which is highly corrosive and can cause severe burns. The solution must be handled under a fume hood at all times. Wear protective clothing including gloves, lab coat, and goggles at all times. Follow all instructions for handling and disposal as indicated in the Material Safety Data Sheet (MSDS) provided.

---

2. Place the microarray horizontally, and add/remove solutions through the portals of the Stripping Cap using a micropipettor with RNase-free pipet tip.
3. Rinse the stripping chamber with 500 µl the Stripping Solution by filling the chamber once and then removing the liquid.
4. Fill the stripping chamber with 500 µl of fresh Stripping Solution. Avoid spilling of the Stripping Solution. If it spills, carefully wipe all liquid from the surface of the stripping clamp with a paper towel/tissue. Air bubbles may form at the top of the chamber, but they will not interfere with stripping (due to the large chamber volume).
5. Hold the assembly horizontally and screw the plugs into the solution portals (see Figure 4).



**Figure 4.** Assembly of the Stripping Clamp with the screw plugs.

6. Place the assembled CustomArray™ Stripping Clamp horizontally into the 65°C incubator, and incubate for 60 minutes.

---

**IMPORTANT!** The CustomArray™ Stripping Clamp assembly should not be rotated, and it should be positioned horizontally to keep the air bubble/s at the top of the chamber.

---

## Washing of ElectraSense™ Microarray after Stripping

1. Remove the CustomArray™ Stripping Clamp from the incubator and place into the fume hood.
2. Let the Clamp cool down for about 5 minutes.
3. Place the assembled CustomArray™ Stripping Clamp horizontally and carefully remove the screw plugs. Aspirate the Stripping Solution by pipetting. Avoid spilling of the Stripping Solution.
4. Wash the chamber with 95% ethanol by filling it once and then removing the liquid.
5. Repeat the ethanol wash (step 4) one more time.
6. Wash the chamber with nuclease-free water by filling it once and then removing the liquid.
7. Repeat the water wash (step 6) one more time.
8. Retain water in the stripping chamber until you are ready to proceed to the next step.

## ElectraSense™ Reading after Stripping

This step is recommended to control the quality of stripping of ElectraSense™ microarrays.

1. Wash the original Hybridization Cap used for hybridization with 95% ethanol and rinse with nuclease-free water. Retain water in the hybridization chamber during preparation of the ElectraSense™ Reader and until electrochemical detection is to be done.
2. Bring an aliquot of TMB Solution to room temperature.

---

**NOTE:** Rinsing with the ElectraSense™ TMB Rinse Solution is not necessary.

---

3. Pipet water out of the stripping chamber. Open the Stripping Clamp, lift the Stripping Cap and remove the microarray. Carefully wipe the contact pads, the area surrounding the semiconductor array, and the back of the slide with a paper towel/tissue.
4. Assemble the ElectraSense™ Microarray with the washed Hybridization Cap and Clamps as described in the ElectraSense™ hybridization protocol.
5. Fill the hybridization chamber with the TMB Solution.

---

**IMPORTANT! Make sure that the next steps (6 to 8) are performed as quickly as possible since the microarray scanning must be performed within 1 minute after addition of the TMB Substrate Solution.**

---

6. Cover both solution portals with adhesive tape.
7. Ensure that all surfaces of the microarray, hybridization cap and clamps are completely dry. If they are not, wipe them with an absorbent tissue.
8. Remove the adhesive tape from the contact aperture, and ensure that the tape covering the solution portals do not obstruct the aperture.
9. Immediately proceed with the microarray scanning, it must be done within 1 minute after addition of the ElectraSense™ TMB Substrate Solution to the hybridization chamber. Read the ElectraSense™ Microarray using the ElectraSense™ Reader instrument as described in the hybridization protocol.
10. Remove the ElectraSense™ microarray from the ElectraSense™ Reader.

---

**NOTE:** If you still observe hybridization signals, the stripping protocol can be repeated.

---

11. If you proceed to immediate re-hybridization or short-term storage, then refer to the section "Preparation of Stripped ElectraSense™ Microarray for Re-hybridization". Alternatively, refer to the section "Storage of Stripped ElectraSense™ Microarray" for long-term storage.

---

**IMPORTANT! Do not store microarrays in the ElectraSense™ TMB Substrate Solution.**

---

## Preparation of Stripped ElectraSense™ Microarray for Re-hybridization

---

NOTE: Proceed to this section if you plan to immediately re-hybridize the stripped microarray, or if the storage time prior to re-hybridization does not exceed several days.

---

1. Pipet the TMB Solution out of the hybridization chamber.
  2. Wash the chamber with 1X PBS by filling it once and then removing the liquid.
  3. Repeat the wash step with 1X PBS two more times for the total of three washes.
  4. Fill the hybridization chamber with 1X PBS, and seal the solution portals with adhesive tape. You can store the microarray assembly for several days at 4°C.
  5. If you proceed to re-hybridization, remove 1X PBS from the hybridization chamber.
  6. Wash the hybridization chamber with nuclease-free water. Remove water from the chamber.
  7. Remove and discard the old Hybridization Cap and Clips.
- 

NOTE: The Stripping Kit contains new hybridization caps for re-use of the stripped microarrays. Discard the used hybridization caps upon completion of the hybridization protocol.

---

8. Immediately wipe the contact pads, the area surrounding the semiconductor array, and the back of the slide with a paper towel/tissue.
9. Assemble the stripped ElectraSense™ microarray with the new Hybridization Cap and Clips. Do **NOT** leave the hybridization chamber empty for any significant length of time, fill the chamber with nuclease-free water for pre-hybridization. Follow the pre-hybridization steps from the corresponding ElectraSense™ Hybridization Protocol.

## Storage of Stripped ElectraSense™ Microarray

---

NOTE: Proceed to this section if you plan long-term storage of the stripped microarrays.

---

1. Pipet the TMB Solution out of the hybridization chamber.
2. Wash the chamber with nuclease-free water by filling it once and then removing the liquid.
3. Repeat the wash step two more times for the total of three washes.
4. Pipet water out of the hybridization chamber. Remove the Hybridization Cap and Clips. Carefully wipe the contact pads, the area surrounding the semiconductor array, and the back of the slide with a paper towel/tissue.
5. Lean the stripped microarray against a tube rack at a 45 degree angle, and leave it to air dry at room temperature until the microarray semiconductor surface looks visibly dry (approximately 30-40 min).
6. The dried microarrays should be placed in a tightly closed box (*i.e.* the original slide retainer box). The dried microarrays can be stored for several weeks in a moisture-free environment (desiccator).