



miRXplore™ Hyb Frames

User manual

Order no. 130-094-454

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1. Description

Components	Five frames in plastic mailer, wooden spatula, 20 seal taps.
Capacity	For five slide hybridizations.
Storage	Store at room temperature, dry and protected from light.

1.1 Background information

Microarrays are a versatile tool for the parallel identification and quantification of different targets like RNA or DNA. The annealing kinetics between the target—here, the labeled RNA- or DNA-molecules in the sample solution—and the fixed DNA probes on a microarray are influenced by several parameters, such as temperature, salt concentration, organic solvents, for example, formamide, incubation volume, and target availability. Due to the limited diffusion rate of the large DNA- or RNA-molecules, target availability can decrease locally during static microarray hybridization. Mixing the target sample, for example, by agitation or even more effectively by active circulation, minimizes such local depletion effects. The miRXplore™ Hyb Frames provide a simple way to agitate microarray incubation with labeled sample solution.

1.2 Applications

The miRXplore Hyb Frames provide the upper and lateral borders of the hybridization space; thus, each framed cover slip defines a sealed hybridization chamber. Subsequently, the researcher is enabled to agitate solutions over the reactive surface of standard microarray on glass slides using standard mixing devices, for example, the MACSmix™ Tube Rotator or a hybridization oven.

▲ **Note:** miRXplore Hyb Frames can only be ordered for the use of miRXplore Microarray Kits.

1.3 Reagent and instrument requirements

- MACSmix™ Tube Rotator (# 130-090-753) or similar agitation instrument
- ▲ **Note:** The continuous tumbling movement of the labeled sample solution over the hybridization area—as in the MACSmix Rotator—is essential for effective hybridizations.

- miRXplore Microarray Kit, including Prehybridization Solution, 2× Hybridization Solution, and 25× Wash Buffers 1 and 2
- Sterile barrier pipette tips
- 0.45 µm sterile filter
- Water bath (42 °C, 50 °C, 98 °C)
- Hot plate (70 °C)
- 50 mL conical tubes
- 2 wash containers for standard microscope glass slides
- Incubation oven
- Scanner for detection of fluorescent microarray signals
- Centrifuge compatible for 2 mL tubes
- Centrifuge or pressured air for microarray drying
- Labeled RNA sample solution (350 µL per microarray)

1.4 Related products

- a-Hyb™ Hybridization Station for automated processing of standard glass slides
- miRXplore Microarray Services
- Bioinformatics Services

2. Protocol

Active agitation of sample solution minimizes non-specific hybridization and improves quality of hybridization signals. Best results are achieved by automated slide hybridization. For further information please contact technical support macstec@miltenyibiotec.de or our website www.miltenyibiotec.com.

For satisfying manual hybridization results, the sample solution should be agitated or mixed continuously! Static hybridization generally results in low signal intensities with a higher degree of non-specific signals and increased detection variance.

The manual hybridization procedure described below takes advantage of the MACSmix Tube Rotator for a continuous tumbling movement of the labeled sample solution over the hybridization area.

▲ The miRXplore Hyb Frames are an extension for the miRXplore Microarray Kits. All buffer components referred to in the protocol are included in the kits.

Before starting

▲ Pre-warm 2× Hybridization Solution to 42 °C.

▲ **Note:** 2× Hybridization Solutions may form precipitates during storage. Warming to 42 °C allows precipitates to dissolve and facilitates pipetting of the viscous solution. Mix the pre-warmed solution thoroughly before pipetting.

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▲ Heat Prehybridization Solution aliquot at 98 °C for 2 minutes, centrifuge briefly, and cool to 42 °C. If necessary, prepare 1× dilutions of the respective Wash Buffers in 0.5L aliquots.

▲ **Note:** Prehybridization Solution and concentrated Wash Buffers may form precipitates when stored. If necessary, redissolve by warming to 50 °C, then cool to room temperature. Make sure that no undissolved particles are present in the washing solution as they may cause background fluorescence.

▲ **Note:** It is recommended to filtrate (0.45 µm filter) all wash buffers prior to use.

▲ The secure adhesion of the hybridization chamber on the glass slide is very important; thus, it is recommended to practice this technique with blank slides before using spotted microarrays.

▲ **Note:** Improper sealing of the hybridization chamber will result in leakage of the hybridization solution and high background signals due to dried areas.

2.1 Microarray and sample preparation

2.1.1 Setting up of the hybridization chamber

1. Take out a miRXplore Hyb Frame to set up a hybridization chamber on the slide. Peel off the thin adhesive foil on the gasket surface of miRXplore Hyb Frame.



2. Place chamber on microarray, aligning the spotted area with the gasket interior.



3. Ensure a firm adherence of the hybridization chamber by pressing gently along the chamber edges with the provided wooden stick. Avoid high pressure that might lead to breakage of the slide.



4. **Warning:** Hot surface! Touching hot surfaces can result in body injury!



For a secure adhesion of the chamber to the slide, place miRXplore Hyb Frame chamber upside-down for at least 20 seconds on a hot surface (about 70 °C).

2.1.2 Filling of the hybridization chamber

1. Pipet 700 µL of Prehybridization Solution through one port of the chamber while allowing air to escape through the other port. Make sure that the surface around both ports is dry.

▲ **Note:** Intentionally, the chamber will not be filled completely. The remaining air supports mixing of the labeled sample solution. Sufficient and correct mixing over the slide edges is essential for the continuous movement of the solution.

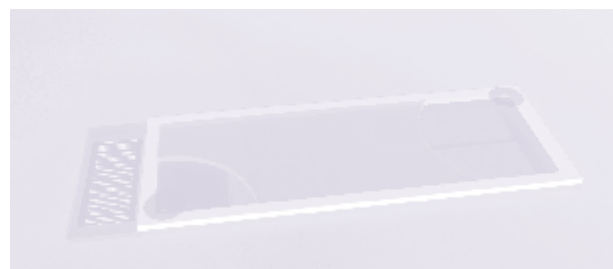


2.1.3 Sealing of the chamber and prehybridization

1. Using forceps, remove seal tab from liner strip and place it carefully over each filling port.



2. Press gently on both seal tabs simultaneously for at least 5 seconds to ensure a secure seal.



3. For constant agitation of the Prehybridization Solution over the slide surface, place microarray gasket with hybridization chamber in a 50 mL tube.

▲ **Note:** It is recommended to fix the microarray inside the tube with few laboratory tissues (e.g. Kim wipes). To ensure a humid atmosphere, pipet 4 mL of ddH₂O over the tissue.

- Place the 50 mL tube into MACSmix Tube Rotator. Please see MACSmix User manual for further instructions.



- Choose medium rotation velocity on the MACSmix Tube Rotator touch pad.

▲ **Note:** The MACSmix Rotator rack should be loaded evenly. For sufficient mixing, the tubes need to be placed in a sloping position into the rack. Overhead rotation (e.g. like in a hybridization oven) is not recommended as sample solution will just move up and down without sufficient mixing.

The MACSmix Tube Rotator runs on rechargeable batteries for at least 24 hours at room temperature. The device can be placed into a refrigerator or an incubator at temperatures between 2 °C and 42 °C. The operation time varies with temperature. Read the MACSmix User manual for details (MACSmix Tube Rotator #130-090-753).

- Place the MACSmix Tube Rotator in an adequate laboratory oven for hybridization at 42 °C. Keep the chambers rotating at 42 °C at least for 10 minutes.

2.1.4 Sample preparation

- Adjust the labeled RNA sample to a volume of 350 µL with nuclease-free water.
- Add 350 µL of a 2× Hybridization Solution, pre-warmed to 42 °C (total sample volume: 700 µL).
- Incubate 700 µL of labeled sample from the previous step at 70 °C for 3 minutes. Centrifuge sample briefly.

2.2 Microarray processing

2.2.1 Hybridization, washing, and drying of microarray

- Take the MACSmix Rotator out of the laboratory oven and let it run until the chambers have reached room temperature.
- Take the microarrays out of the 50 mL tubes.
- Place the microarray on a flat surface. Be sure that the slide surface is dry and dust-free.
- Remove seal tabs from each filling port.
- Remove 700 µL of Prehybridization Solution from one port using a pipette.

▲ **Note:** Residual amounts of Prehybridization Solution may stay in the chamber.

- Pipet 700 µL of labeled sample solution through one port of the chamber while allowing air to escape through the other port. Make sure that the surface around both ports is dry.

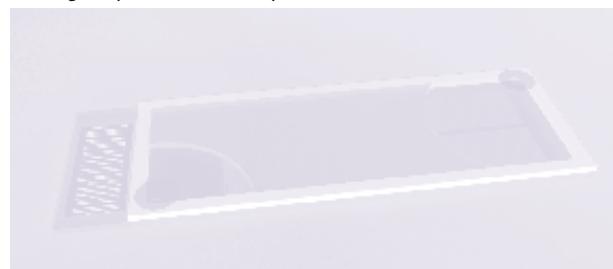
▲ **Note:** Intentionally, the chamber will not be filled completely. The remaining air supports mixing of the labeled sample solution. Sufficient and correct mixing over the slide edges is essential for the continuous movement of the solution.



- Using forceps, place seal tabs centred over each filling port.



- Press gently simultaneously for at least 5 seconds on both tabs.



- Place microarray gasket with hybridization chamber in a 50 mL tube.

▲ **Note:** It is recommended to fix the microarray inside the tube with few laboratory tissues (e.g. Kim wipes). To ensure a humid atmosphere, pipet 4 mL of ddH₂O over the tissue.

- Place the 50 mL tube into MACSmix Tube Rotator; please see MACSmix User manual for further instructions.



- Choose slow rotation velocity on the MACSmix Tube Rotator touch pad.

▲ **Note:** The MACSmix Tube Rotator rack should be loaded evenly. For sufficient mixing, the tubes need to be placed in a sloping position into the rack. Overhead rotation (e.g. like in a hybridization oven) is not recommended as sample solution will just move up and down without sufficient mixing.

The MACSmix Tube Rotator runs on rechargeable batteries for at least 24 hours at room temperature. The device can be placed into a refrigerator or an incubator at temperatures between 2 °C and 42 °C. The operation time varies with temperature. Read the MACSmix User manual for details (MACSmix Tube Rotator #130-090-753).

- Place MACSmix Rotator in an adequate laboratory oven for hybridization. Incubate for minimal 14 hours at 42 °C.

▲ **Note:** With the continuous tumbling rotation of the microarray chambers inside the MACSmix Rotator, the chamber content is moved constantly in circles over the spotted area. The inserted air helps distributing the labeled nucleic acids as evenly as possible across the hybridization area.

2.2.2 Postprocessing

- Fill a wash container with fresh Wash Buffer 1 at room temperature.

▲ **Note:** In order to ensure a correct workflow it is recommend to work with two wash containers.

- Remove microarray from the hybridization oven.

- Grasp the edge of chamber firmly and peel off the miRXplore Hyb Frame from the microarray.

▲ **Note:** Take care to minimize the formation of splashes or droplets, wear goggles as a precaution.

- Place microarray **immediately** in the wash container filled with 50 mL of fresh 1× Wash Buffer 1 for 5 minutes at room temperature.

- Repeat step 4 using fresh 1× Wash Buffer 1.

- Wash microarray with 50 mL of fresh 1× Wash Buffer 2 for 5 minutes at room temperature.

- Repeat step 6 using fresh 1× Wash Buffer 2.

- Dip microarray three times quickly into ddH₂O (room temperature).

▲ **Note:** This final wash step ensures that no remaining salt residues are left on the slide.

- Dry microarray by centrifugation at 500×g for 3 minutes, or with compressed, dry air (or inert gas like He, N₂) from a clean supply, and store it in a dust-free hybridization cassette in the dark.

▲ **Note:** If using compressed gas, dry slide starting from the opposite side of the barcode label towards barcode label.

2.2.3 Scanning

Scan the microarrays according to the instruction of the available scanning instrument.

▲ **Note:** In case of repeated scanning of the same microarray, scanner-derived bleaching may occur, mainly in the Cy5 channel. This is also dependent on additional environmental factors, e.g. ozone¹.

4. Reference

- Fare, T. L. *et al.* (2003) Effects of atmospheric ozone on microarray data quality. *Anal. Chem.* 75: 4672–4675.

All protocols and data sheets are available at www.miltenyibiotec.com.

Warranty

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