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a-Hyb™ Hybridization Buffer Kit User manual

Order no. 130-093-428



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1. Description

1.1 Components and sizes

- 2×2 mL PIQOR™ 2× Hybridization Solution,
- 2×50 mL PIQOR Prehybridization Solution, ready-to-use (reorder# 130-091-776)
- 2×200 mL Wash Buffer 1, ready-to-use
- 2×200 mL Wash Buffer 2, ready-to-use
- 1× User manual

Storage

Store PIQOR 2× Hybridization Solution and PIQOR Prehybridization Solution protected from light at -20 °C. Store the Wash Buffers at room temperature (RT). The kit content is stable for six months.

1.2 Kit capacities

For up to 20 microarray hybridization experiments.

The cover photo shows a replica of the DNA model built in 1953 by James D. Watson and Francis Crick at the Cavendish Laboratory in Cambridge. This model is located at Heureka, the Finnish Science Centre. Photography by Alexander Budde. © Miltenyi Biotec GmbH, Germany. Detailed information on the history of the Watson-Crick model can be found in: de Chadarevian, S. (2003) Relics, replicas and commemorations. Endeavour 27: 75–79.

1.3 Reagents and instrument requirements

- Double-distilled water (ddH₂O)

For hybridization in the a-Hyb™ Hybridization Station

- a-Hyb™ Hybridization Station and accompanying accessories
- (Optional) 50% formamide in ddH₂O for automatic denaturation
- (Optional) 96% ethanol (EtOH) for manual denaturation
- (Optional) Empty glass slides when using less than four Microarrays per experiment
- (Optional) Isopropanol for automated drying of slides inside a-Hyb Station
- (Optional) Wash tray for microscopic slides for drying slides manually
- (Optional) Nitrogen or pressured air source or centrifuge for drying of slides outside of an a-Hyb Station

For manual hybridization (PIQOR™ HybChamb)

- 96% ethanol (EtOH) for manual denaturation
- (Optional) PIQOR™ HybChamb for manual hybridization
- PCR-cycler, waterbath, or hybridization oven
- Wash tray for microscopic slides for washing and drying of slides manually
- Nitrogen or pressured air source or centrifuge for drying of slides

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2. Protocol**2.1 Preparations**

1. Heat Prehybridization Solution to 98 °C for 2 minutes and cool down to 42 °C before usage.
2. If the Wash Buffers contain precipitates, heat the buffers to 50 °C until the precipitate dissolves and cool down to room temperature before usage. In case the precipitates do not dissolve after incubation at 50 °C the solutions should be sterile-filtered before use with 0.2–0.45 µm filters.
3. Pre-heat water bath or heating block to 65 °C.

For hybridization in the a-Hyb™ Hybridization Station

Prehybridization Solution and Wash Buffers are ready to use. No dilution or addition of further substances is necessary.

The Hybridization Solution is double-concentrated. Dilute your sample with distilled water or concentrate the sample in a speedvac to half of the final hybridization volume. Add the same volume of 2× Hybridization Solution. For the hybridization in the a-Hyb™ Station, dilute or concentrate your sample to 100 µL and add 100 µL of 2× Hybridization Solution to a final hybridization volume of 200 µL.

Connect buffer bottles with the corresponding tubes for automated medium supply. The wash buffer bottles are compatible with the a-Hyb Bottle caps. Simply remove the caps of the wash buffer bottles and connect the a-Hyb Tube together with the a-Hyb Bottle cap to the original wash buffer bottle.



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Connect the medium tubes to the following solutions:

Medium tube 3: Prehybridization Buffer

Medium tube 1: Wash Buffer 1

Medium tube 2: Wash Buffer 2

(Optional) For automated drying – Medium tube 7: Isopropanol

(Optional) For automated denaturation – medium tube 4:

50% formamide in ddH₂O

Medium tube 8: ddH₂O

▲ **Note:** Please make sure that this usage of tubes corresponds to the selected a-Hyb™ Protocol, see section 3.1 for an example of an a-Hyb Hybridization Station protocol.

▲ Before starting a hybridization experiment it is recommended to remove residual buffer or water from the external a-Hyb Medium Tubes. Disconnect the tubes and let residual water run out or start the storage protocol with all tubes in an empty bottle.

▲ For automatic buffer supply in the a-Hyb Hybridization Station, about 5 mL of buffer is required per step and activated microarray position. If all four positions are selected, approximately 20 mL per step are necessary (20 mL of Prehybridization Solution and 40 mL of Wash Buffer for two wash cycles). For manual sample application in the a-Hyb Station, a volume of 200 µL labeled sample per position is needed.

Manual hybridization (PIQOR™ HybChamb)

Prehybridization Solution and Wash Buffers are ready to use. No dilution or addition of further substances is necessary. For cDNA microarrays, pre-heat Wash Buffer 1 and Wash Buffer 2 to 50 °C.

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The Hybridization Solution is double-concentrated. Dilute your sample with distilled water or concentrate the sample in a speedvac to half the hybridization volume. Add the same volume of 2× Hybridization Solution.

For manual coverslip hybridization dilute or concentrate your sample to 20 µL and add 20 µL 2× Hybridization Solution to a final hybridization volume of 40 µL.

2.2 (Optional) Denaturation of microarrays

(Optional) To denature probes on microarray, see section 3.3 for manual or automated denaturation protocols.

▲ For automated denaturation it is necessary to insert two extra steps into the hybridization protocol, see section 3.3.1.

▲ Depending on the experimental goal, probe denaturation might be omitted. Skipping of the denaturation step might lead to slightly lower signal intensities and specificities; background fluorescence should not be increased.

2.3 Hybridization, washing, and drying

▲ Prehybridization of microarrays significantly reduces the background fluorescence and is therefore strongly recommended.

Hybridization in the a-Hyb™ Hybridization Station

Please consult the a-Hyb™ Hybridization Station user manual for detailed information on installation, setup, and usage of the a-Hyb Instrument.



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Start of process

1. Start the a-Hyb Hybridization Station: Switch power button on.
2. Switch to the hybridization screen designated by the blue area.
3. Select slide positions to be used for hybridization.
4. Select the appropriate protocol e.g. PIQOR™ Protocol.

▲ **Note:** Select **View protocol** to ensure that the protocol is unchanged and the buffer selection corresponds to the tube numbers selected. See appendix for further information on a-Hyb™ Protocols. Check whether the protocol contains the step Dry and if necessary adapt the protocol according to your experimental setup (remove/add Dry step).

5. Switch to the hybridization screen and press **Start** to begin the hybridization process.
6. Load microarrays in a-Hyb Slide Carrier, and place plain glass slides in unoccupied positions.

7. Place a-Hyb Sealing Plate on top of a-Hyb Slide Carrier.

▲ **Note:** Shortly rinse the a-Hyb Sealing Plate with water before (and after) use.

8. Place a-Hyb Slide Carrier together with sealing plate into drawer and press **Continue**. The drawer moves into the instrument and a lift mechanism will raise the slides and seal the hybridization chambers. The hybridization process will start automatically.
9. In the meantime, pre-heat cDNA samples to 65 °C for 2 minutes.

Sample loading

1. After prehybridization, the software will ask to press continue before adding the sample: Press **Continue** when you are ready to add the sample.

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2. The software will immediately ask for sample application: Pipette 200 µL of labeled cDNA sample in the respective reservoir and press **Continue**.
3. Follow the instructions on the instrument screen and apply further samples for all selected positions—repeat step 1 and 2 for all samples—when prompted.

Washing

All wash steps will be performed automatically.

Option 1: Manual drying

1. Prepare slide-staining dish: Fill it with Wash Buffer 2 or one dish with Wash Buffer 2 and a second one with ddH₂O.
2. At the end of the process the software will ask whether it should cool down to 25 °C. Press **No**. Press **Continue** to stop all processes and open the instrument.
3. Immediately remove the sealing plate and place the slide carrier on the slide loading device to take out the slides.
4. Immediately transfer slides to a slide rack within a slide-staining dish containing Wash Buffer 2 or ddH₂O.

▲ **Note:** It is important to immediately transfer the hybridized microarrays into the vessel with Wash Buffer 2 or ddH₂O after opening the instrument. Drying of the microarrays at this step might lead to elevated background fluorescence.
5. Dip the slides twice in dish with ddH₂O.
6. Dry microarray by using either nitrogen/compressed air or by centrifugation at 1500 × g for 3 minutes.
7. The microarrays are ready to be scanned.



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Option 2: Automatic drying

Drying will be done automatically at the end of the process, see section 3.2. for further information.

Manual hybridization (PIQOR™ HybChamb)

For manual hybridization, care should be taken not to let the microarray dry out as hybridization solutions can evaporate at the recommended hybridization temperature. Therefore, the use of a humidified hybridization cassette such as the PIQOR™ HybChamb is recommended.

The PIQOR HybChamb is a small-sized, watertight chamber for four microarrays. It can be used for hybridization in a water bath or hybridization oven or on a MJ Research Thermocycler with a temperature-controlled lid. To humidify the chamber, water is applied in the respective cavities without risk of the hybridization solution being diluted or contaminated.

The small racks in the PIQOR HybChamb allow easy insertion and removal of microarrays, spacers avoid cross-contamination between different microarrays.

▲ The microarrays should be placed in a horizontal position during all prehybridization and hybridization steps.

Prehybridization

1. Apply 40 µL of PIQOR Prehybridization Solution onto the microarray in the spot area.
2. Place the coverslip over the microarray.
3. Place microarray in a humidified hybridization cassette (e.g. PIQOR HybChamb.)

4. Seal the cassette and incubate in a water bath, a hybridization oven, or a PCR Cycler at 42 °C for oligonucleotide microarrays or at 65 °C for cDNA microarrays for at least 30 minutes.

▲ **Note:** Make sure that the hybridization cassette is properly sealed to prevent the hybridization reaction from drying out.

Hybridization

1. Cool the hybridization cassette to 25 °C by leaving it at room temperature for at least 20 minutes.
2. Incubate 40 µL of labeled sample at 65 °C for 2 minutes.
3. Remove the microarray from the cassette and carefully remove the coverslip.
4. Centrifuge sample briefly and apply immediately to the spot area of the microarray.

▲ **Note:** Simply apply the labeled sample without removing residual Prehybridization Solution. Do not wash the microarray and do not allow it to dry.
5. Place fresh coverslip on to the microarray.

▲ **Note:** Do not reuse the coverslip from the prehybridization step.
6. Place in a humidified hybridization cassette. Seal the cassette and incubate overnight (minimum 6 hours) in a hybridization oven, a water bath, or in an appropriate PCR cycler at 42 °C for oligonucleotide microarrays or at 65 °C for cDNA microarrays.

▲ **Note:** Make sure that the hybridization cassette is properly sealed to prevent the hybridization reaction mix from drying out.

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Washing

1. Remove microarray from the hybridization cassette and place it immediately in the wash container filled with 50 mL Wash Buffer 1 for 5 minutes at room temperature for oligonucleotide microarrays or at 50 °C for cDNA microarrays. If the coverslip does not slide off the microarray upon placing it into the Wash Buffer 1 remove the coverslip by gently pushing the coverslip (touching only the surface) a few millimeters over the border of the glass slide and removing it with forceps, .

▲ **Note:** Do not allow the microarray to cool down before placing it in Wash Buffer 1.

2. Repeat step 1: Wash the microarray with 50 mL Wash Buffer 1 for 5 minutes at room temperature for oligonucleotide microarrays or at 50 °C for cDNA microarrays.
3. Wash microarray with 50 mL Wash Buffer 2 for 5 minutes at room temperature for oligonucleotide microarrays or at 50 °C for cDNA microarrays.
4. Repeat step 3: Wash microarray with 50 mL Wash Buffer 2 for 5 minutes at room temperature for oligonucleotide microarrays or at 50 °C for cDNA microarrays.

Drying

1. Dip microarray three times quickly into distilled water at room temperature.

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

2. 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 in a dust-free hybridization cassette.

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

3. The microarray is ready for scanning.

3. Appendix**3.1 Example of an a-Hyb™ Hybridization Station protocol**

▲ For further application and protocol examples please see also the a-Hyb™ Protocol booklet. Outlined below is the PIQOR™ Protocol that is also stored in the a-Hyb Hybridization Station.

Step	Medium	Temperature (°C)	Time (min)	Pump speed (mL/min)	Cycles
Incubation	3, 3, 3, 3	63, 63, 63, 63*	5	1.0	–
Incubation	M, M, M, M	63, 63, 63, 63*	960**	1.0	–
Wash	1, 1, 1, 1	50, 50, 50, 50	1	1.0	2
Wash	2, 2, 2, 2	35, 35, 35, 35	1	1.0	2
End					

* The temperature for prehybridization and hybridization has to be adapted to probe length. We recommend 42 °C for oligo probes and 63 °C for cDNA probes but the best temperature should be determined experimentally.

** The hybridization time can be reduced to 6 hours due to the active circulatory system in the a-Hyb™ Station.

Automated buffer supply

Media 1: Wash Buffer 1 via tube 1

Media 2: Wash Buffer 2 via tube 2

Media 3: Prehybridization Solution via tube 3

Media 8: ddH₂O via tube 8

Sample application

Media M: Labeled sample via sample reservoir. Load sample only before instrument prompts for it during the hybridization process (at the beginning of the second incubation step).

3.2 Automatic drying

For automatic drying, an additional step has to be added to the PIQOR™ Protocol: Insert step Dry as the last step to the protocol.

Step	Medium	Temperature (°C)	Time (min)	Pump speed (mL/min)	Cycles
Incubation	3, 3, 3, 3	63, 63, 63, 63	5	1.0	–
Incubation	M, M, M, M	63, 63, 63, 63	960	1.0	–
Wash	1, 1, 1, 1	50, 50, 50, 50	1	1.0	2
Wash	2, 2, 2, 2	35, 35, 35, 35	1	1.0	2
Dry					
End					

Additional buffer needed for automated buffer supply

Media 1–3 and M: see above in section 3.1.

Media 7: Isopropanol

Media 8: ddH₂O

3.3 Denaturation

3.3.1 Automatic denaturation during PIQOR™ Protocol

For automatic denaturation, insert an extra incubation and wash step at the beginning of the protocol.

1. Incubate microarrays at 75 °C for 2 minutes with a pump speed of 1.5 mL/min, using a solution of 50% formamide in distilled water (medium bottle 4).
2. Wash slides with distilled water at 75 °C for 2 minutes with a pump speed of 1.5 mL/min. Two cycles should be performed to wash away the formamide and any unbound DNA molecules.

Step	Medium	Temperature (°C)	Time (min)	Pump speed (mL/min)	Cycles
Incubation	4, 4, 4, 4	75, 75, 75, 75	2	1.5	–
Wash	8, 8, 8, 8	75, 75, 75, 75	2	1.5	2
Incubation	3, 3, 3, 3	63, 63, 63, 63	5	1.0	–
Incubation	M, M, M, M	63, 63, 63, 63	960	1.0	–
Wash	1, 1, 1, 1	50, 50, 50, 50	1	1.0	2
Wash	2, 2, 2, 2	35, 35, 35, 35	1	1.0	2
End					

Buffer supply

As in section 3.1. Also:

Media 4: 50% formamide in ddH₂O

Media 8: ddH₂O

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3.3.2 Manual denaturation

1. Place a ddH₂O-filled glass beaker in a water bath set to 95 °C. Use an empty glass slide to check that the water-level stays below the barcode label. The microarrays are vertically placed in the glass beaker. Heat the microarrays in ddH₂O at 95 °C for 2 minutes. Avoid overboiling.
 - ▲ **Note:** This step denatures the double-stranded DNA molecules on the glass slide.
2. Immediately transfer the microarrays into 96% EtOH for at least 30 seconds. Avoid contact of the barcode label with 96% EtOH.
3. Dry slides by centrifugation for 3 minutes at 500 × g, or with pressured gas from a clean supply.
 - ▲ **Note:** If using pressured gas, dry slide starting from the opposite site of the bar code towards bar code label.
4. Place in a dust-free slide holder and proceed to section 2.3, step 3.

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