Hyb-Seal™ Incubation Chambers

for Sealing Reactions on Slides

Hyb-Seal incubation chambers provide vapor-tight sealing for FISH, in situ PCR, and PRINS applications, and allow repeatable access to the reaction fluid. The adhesive chambers are placed over the specimen area and fastened to the slide. Reaction mix is added through either of the small holes in the coverslip, and then both holes are sealed with small adhesive plastic dots. After cycling, the adhesive dots can easily be removed, and the reaction mix can be retrieved. Dots can be reattached if necessary. Hyb-Seal chambers can be UV-treated, but autoclaving is not recommended. These seals are compatible with MJ-line and other horizontal-format slide thermal cyclers.

Directions for Use

1. Remove chamber from plastic sheet and position adhesive side down over the desired area of the slide. Sample should not be positioned directly under the holes in the Hyb-Seal chamber.
2. Press along the blue border to fasten chamber to slide. To ensure complete adhesion, rub firmly back and forth over the frame with a hard object.
3. Pipet the appropriate volume of reagent into the chamber through either of the holes (the other acts as a vent).
4. Place an adhesive dot over each hole and press down gently. The dots will adhere to the wet surface.
5. The slide assembly is now ready to use.

Hyb-Seal Chambers 100 chambers and 200 sealing dots per package

SLH-2001	Hyb-Seal Chambers, 20 x 19 mm enclosed area, 165 µl capacity
SLH-4001	Hyb-Seal Chambers, 20 x 37 mm enclosed area, 355 µl capacity
SLH-6001	Hyb-Seal Chambers, 20 x 63 mm enclosed area, 465 µl capacity

Alternative Slide-Sealing Products

SLF-0201	Frame-Seal™ Chambers, 9 x 9 mm, 25 µl capacity
SLF-0601	Frame-Seal Chambers, 15 x 15 mm, 65 µl capacity
SLF-1201	Frame-Seal Chambers, 17 x 28 mm, 125 µl capacity
SLF-3001	Frame-Seal Chambers, 19 x 60 mm, 300 µl capacity
SLR-0101	Self-Seal™ Reagent, 2x, 5 x 1 ml, sufficient for 200 x 50 µl reactions or 600 x 15 µl reactions

Note: Hyb-Seal and Frame-Seal chambers are not intended for use in combination with Self-Seal reagent.

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Tips for Best Results

- Ensure that the slide surface that will contact the Hyb-Seal chamber is clean and dry
- Hold the chamber at its edges to reduce contact with the exposed adhesive
- Firmly press with a hard object around the blue edges of the Hyb-Seal chamber, eliminating any air bubbles trapped between the adhesive and the glass slide
- Allow the adhesive to set completely by applying the chamber the day before use or by incubating at 95°C for 5 min
- Assemble and mix reaction components in a tube, creating excess to prevent air bubbles; for example, use 175 µl of reaction mix for the 165 µl capacity Hyb-Seal chamber. De-gas the mixture for 5 min in a vacuum chamber before pipetting into the chamber
- The dot is easily lifted and removed with a razor blade post-incubation, so the reaction fluid can be retrieved, changed, or supplemented. Dot can be reattached if necessary

Protocol Considerations

- Optimize the reaction conditions, especially annealing temperature, in a tube before optimizing in the slide format. Use optimized tube conditions as the starting point for further optimizing conditions in the slide format. Optimize without tissue present first, and then with tissue
- To prevent binding of proteins and nucleic acids to the glass slide, add 0.05–0.1% BSA or other carrier protein to the reaction mix
- When a tissue is first used, optimize the protease digestion steps before adding the amplification steps. Optimal conditions are those that provide enough digestion to allow a simple in situ hybridization to work effectively, while still adequately preserving cell morphology. Optimize the protease concentration and digestion time for each new tissue type and each new lot of protease
- To minimize tissue damage during PCR, minimize the total number of cycles performed (try doing 15–20 cycles) and the total time spent above 90°C. The PCR needs to increase the amount of target to only a few hundred or a few thousand copies — enough to be detected by probe hybridization
- Don't use one-step methods that incorporate biotin–dNTPs into the PCR mix. These often lead to nonspecific background. Instead, amplify in one step, wash, then hybridize the probe in a separate step

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