

For automated Fluorescence *in situ* Hybridization (FISH) using *ZytoMation* FISH probes on Leica BOND-III or BOND-MAX.

1) Preparatory Steps (Day 1)

- Start Computer, Label Printer, BOND-Stainer
- Transfer Probe to Titration Kit (Leica OPT9049)
- Prepare Proteinase K dilution (Leica AR9551)
- Equip BOND FISH Kit (DS9636) with Probe and Enzyme Tray with labeled specimen slides

(Day 2)

- Prewarm DAPI/DuraTect™ -Solution to RT
- Prepare Ethanol series (70%, 90%, 100%)

2) Protocol Setup (Day 1)

- Open „Slide Properties“
- Switch to „ISH“ and choose a marker
- *FISH Protocol D
- *Dewax
- HIER with ER2 (Leica AR9640) 25 min 100°C
- Digestion with Enzyme Pretreatment Kit (Leica AR9551), pretest required x min 37°C
- *Denaturation (10min)
- *ISH Hybridization (12Hr)

This is a condensed protocol for *ZytoMation*® probes and should not replace the manual included with each product!
Please refer to the current manual of the respective probe in use!

3) Detection (Day 2)

- Dehydrate 70%, 90%, 100% Ethanol each 1 min RT
- Air drying
- Apply DAPI/DuraTect™ -Solution, 20 µl
- Cover with coverslip, seal
- Incubate (protected from light) 15 min RT
- Evaluate slides

Required Leica Products	Prod. No.
• Dewax Solution	AR9222
• Epitope Retrieval Solution 2 (EDTA)	AR9640
• Wash Solution 10X Concentrate	AR9590
• Universal Covertile	S21.4611 (160 pcs) S21.2001 (100 pcs)
• Enzyme Pretreatment Kit	AR9551
• Titration Kit	OPT9049
• Bond FISH Kit	DS9636

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