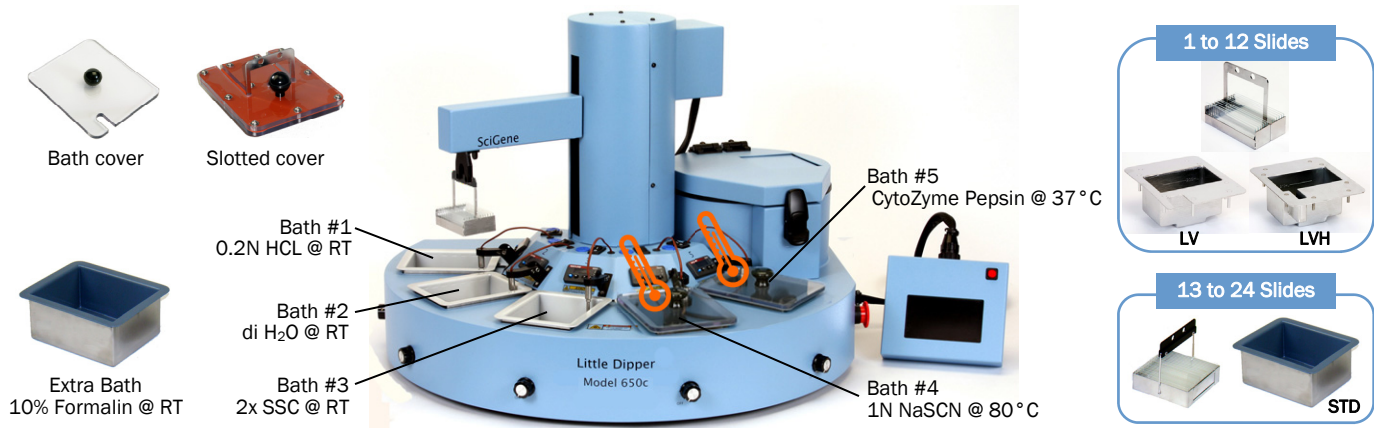


Automated Processing of FFPE Tissues for FISH Analysis



NOTE: This method is derived from the manual procedure used with the Abbott Paraffin Pretreatment Kit. Since many variations are possible, this method should be considered only as an example. Custom protocols for your specific process are easily programmed.

Baths and Accessories

Table 1 lists the number and types of baths, racks and bath covers for setting up the instrument for processing batch sizes of 1-12 and 13-24 slides.

Table 1. Bath and Accessories for Different Batch Sizes

Items Required for 1 to 12 Slides	Catalog #	Qty
Standard bath with stir bar (STD)	1080-10-1	1
Low volume bath with stir bar (LV)	1080-10-2	3
Low volume, heatable bath with stir bar (LVH)	1080-10-5	1
12-position slide rack*	1080-20-1	2
Bath cover	1080-20-0	2
Slotted cover for 12-position rack	1080-12-2	1
Items Required for 13 to 24 Slides	Catalog #	Qty
Standard bath with stir bar (STD)	1080-10-1	6
24-position slide rack*	1080-20-5	2
Bath cover	1080-20-0	2
Slotted cover for 24-position rack	1080-12-4	1

*Requires matching sized centrifuge buckets

Reagents

- 0.2 N HCL — dilute from concentrate with diH2O
- 1N NaSCN — ready-to-use (SciGene cat. #2022-11-X)
- CytoZyme Stabilized Pepsin (SciGene cat. #2022-00-X)
Dilute 1:50 v/v in CytoZyme Reaction Buffer (cat. #2022-10-X)
- 2X SSC — dilute from 20X concentrate with diH2O
- 10% neutral buffered formalin

Consult Table 2 for the volume of each reagent to be prepared for processing different slide batch sizes.

Table 2. Bath Setup for FFPE Slide Processing

Bath Position	Reagents for 1 to 12 Slides	Bath Type	Volume	Temp (°C)
1	0.2N HCL	LV	270	RT
2	di H ₂ O	LV	270	RT
3	2x SSC	STD	670	RT
4	1N NaSCN	LVH	290	80°
5	CytoZyme Pepsin	LVH	290	37°
Extra	10% Formalin	LV	270	RT
Bath Position	Reagents for 13 to 24 Slides	Bath Type	Volume	Temp (°C)
1	0.2N HCL	STD	670	RT
2	Di H ₂ O	STD	670	RT
3	2x SSC	STD	670	RT
4	1N NaSCN	STD	670	80°
5	CytoZyme Pepsin	STD	670	37°
Extra	10% Formalin	STD	670	RT

Instrument Programming and Setup

1. Using the touchscreen, name and create the protocol shown in Table 3. Consult the **Little Dipper User Manual** for instructions on creating protocols with a user controlled programmed pause.
2. Referencing Table 2, place required baths in the indicated positions on the instrument. An extra bath is kept ready to replace the bath #1 for the final formalin fixation step.
3. Fill each bath with its specified reagent to the max fill line and set stir bar rotation speed to achieve a gentle vortex.
4. Turn on power to heat the baths in positions 4 and 5. Set the temperature of bath #4 to 80°C and bath #5 to 37°C. Place a bath cover over each to prevent evaporation.

Continued on next page...

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Table 3. Sample Protocol for FFPE Slide Processing (FFPE-1)

Step #	Bath #	Agitation (cpm)	Time (sec)	Pause (sec)	Drip Time(sec)
1	#1	150	1200	0	0
2	#2	150	180	0	0
3	#3	150	180	9999	0
— Programmed Pause —					
4	#4	0	1800	0	0
5	#3	150	600	9999	0
— Programmed Pause —					
6	#5	150	600	0	0
7	#3	150	600	9999	0
— Programmed Pause —					
8	#1	150	600	0	0
9	#3	150	600	0	0

Deparaffinization

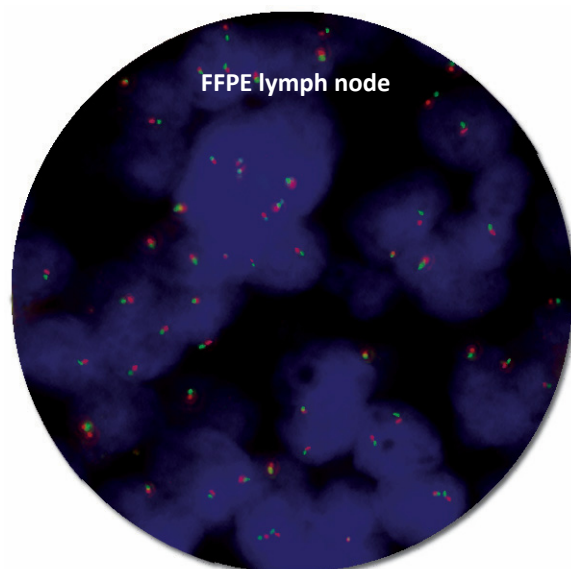
Load slides to be processed in the slide rack (12 or 24 position as needed) and process using your standard protocol (xylene, Hemo-D etc) in a fume hood followed by air drying.

Load Slides /Run Protocol

1. Check that all baths on the instrument are filled properly and stir bars are providing good mixing action. Verify that bath #4 is 80°C and bath #5 is 37°C. Keep bath covers in place until later in the protocol.
2. Load the slide rack containing the de-waxed slides and start the protocol.
3. At the completion of step 3, the instrument will pause and provide an audible signal. Remove the bath cover from pretreatment bath #4 (Fig. 1). Release the pause by touching the touch screen. When the rack is submerged in pretreatment solution, slide the slotted cover around the handles of the rack.

Fig 1. Use slotted cover during processing.
4. At the completion of the pretreatment (step 5), the instrument will again pause and sound an alert. Slide out and remove the slotted cover from around the rack handles and remove the bath cover on bath #5. Resume the protocol by touching anywhere on the touchscreen.
5. After protease treatment (step 7), the instrument will pause again. Replace the bath in position #1 with the extra bath containing formalin. Resume protocol by pressing the touch screen.
6. At the completion of the protocol the slide rack will be suspended above bath #1. Remove the slide rack from the instrument and dry slides on a slide warmer at 45-50°C.

— Proceed to probe hybridization. —



FISH images from slides processed on the Little Dipper Processor for FISH.

SciGene