METHODS

Automated Processing of FFPE Tissues for FISH Analysis

**Equipment Configuration**

- **Little Dipper Processor** for FISH.
  - SciGene cat. #1080-70-1 (115V) / 1080-70-2 (230V)
- **1x Standard, coated bath (STD-C)** for NaSCN with stir bar.
  - SciGene cat. #1080-10-7
- **4x Low volume heatable bath, 275 ml with stir bar. (LVH)**
  - SciGene cat. #1080-10-5
- **2x Slide rack, 12 position for 3 inch slides.**
  - SciGene cat. #1080-20-1
- **1x Bath cover** — for pre-heating NaSCN only.
  - SciGene cat. #1080-12-1

**Reagents Needed**

- **CytoZyme Stabilized Pepsin** (SciGene cat. #2022-00-3)
- **CytoZyme Stabilized Pepsin HC** [high concentration]
  - SciGene cat. #2022-03-3
- **CytoZyme Reaction Buffer** (SciGene cat. #2022-10-3)
- **1M NaSCN** — (SciGene cat. #2030-00-2)

**Table 1. CytoZyme Dilutions Equivalent to Abbott Kits.**

<table>
<thead>
<tr>
<th>Abbott Kit</th>
<th>CytoZyme Type</th>
<th>Pepsin (ml)</th>
<th>Buffer (ml)</th>
<th>Total Bath Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I - CytoZyme Peps -OR-</td>
<td>5</td>
<td>270</td>
<td>275</td>
<td></td>
</tr>
<tr>
<td>II CytoZyme HC Peps日益</td>
<td>0.5</td>
<td>274</td>
<td>275</td>
<td></td>
</tr>
<tr>
<td>IV CytoZyme HC Peps日益</td>
<td>1.5</td>
<td>268.5</td>
<td>275</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Adjust CytoZyme concentration or digestion times for variations in sample thickness and other factors. Run test slides to determine optimum conditions.

**Instrument Setup**

1. Turn on main power to the instrument (right side, at back).
2. Insert baths as shown in Figure 1. Place stir bars in Baths 2-5.
3. Fill Bath 2 (STD-coated bath) with 670ml of NaSCN.
4. Turn on Bath 2 controller and set temperature to 80°C. Adjust stir bar rotation until a gentle vortex is formed.
5. Place a cover over Bath 2 to minimize evaporation.
6. Prepare a CytoZyme solution by adding pepsin and buffer equivalent to your Abbott kit in Bath 4 (Table 1).
7. Turn on Bath 4 controller and set temperature to 37°C. Adjust stir bar rotation until a gentle vortex is formed.
8. Add DI H2O to fill line in Baths 3 and 5. Adjust stir bar rotation until a gentle vortex is formed.
9. Wait for bath temperatures to stabilize (15-20 min).

**Table 2. Bath Setup for FFPE Tissue Processing.**

<table>
<thead>
<tr>
<th>Bath</th>
<th>Reagents</th>
<th>Bath Type</th>
<th>Volume (ml)</th>
<th>Temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>none</td>
<td>LVH</td>
<td>—</td>
<td>RT</td>
</tr>
<tr>
<td>2</td>
<td>NaSCN</td>
<td>STD-C</td>
<td>670</td>
<td>80*</td>
</tr>
<tr>
<td>3</td>
<td>DI H2O</td>
<td>LVH</td>
<td>275</td>
<td>RT</td>
</tr>
<tr>
<td>4</td>
<td>CytoZyme + Buffer</td>
<td>LVH</td>
<td>275</td>
<td>37*</td>
</tr>
<tr>
<td>5</td>
<td>DI H2O</td>
<td>LVH</td>
<td>275</td>
<td>RT</td>
</tr>
</tbody>
</table>

**Deparaffinization**

Perform deparaffinization of the slides to be processed on the Little Dipper using your laboratory’s standard procedure.

Continued on next page...
Automated Processing of FFPE Tissues for FISH Analysis

**Load Slides / Run Protocol**

1. Using the Little Dipper Processor touch screen, create the FFPE protocol in Table 3. Consult the Little Dipper User Manual for instructions.

2. After temperature has stabilized in Baths 2 and 4, load a 12-position rack with dewaxed slides and place near Bath 1.

3. Remove the cover on Bath 2 and set aside.

4. Start the FFPE protocol from the touchscreen and load the rack in Bath 1. The instrument will perform the protocol and stop with the rack above Bath 5.

5. Push the tab on the gripper paddle -OR- use the Advance Settings | Open Gripper command on the touch screen to release the rack. Slides are now ready for probe hybridization.

---

**Table 3. Little Dipper FFPE Protocol for DeWaxed Samples**

<table>
<thead>
<tr>
<th>Step</th>
<th>Bath</th>
<th>Agitation (cpm)</th>
<th>Time (sec)</th>
<th>Pause (sec)</th>
<th>Drip (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0</td>
<td>720</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0</td>
<td>180</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>0</td>
<td>1200</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0</td>
<td>180</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

---

**Fig 2. FISH analysis of FFPE tissue processed on the Little Dipper instrument using SciGene CytoZyme Stabilized Pepsin and pretreatment reagents.**