INTENDED USE

Wright Stain is intended for use in staining blood films or bone marrow films. Solutions are for “In Vitro Diagnostic Use.”

Wright Stain is a modified Romanowsky stain intended for differentially staining the cellular elements of blood. When blood films are treated as herein described, the white blood cell nucleus and cytoplasm take on characteristic blue or pink coloration. The purified dyes in the Sigma-Aldrich formulations of Wright Stain eliminate inconsistent staining and yield reproducible lot-to-lot chromogenic responses.

PROCEDURE

SPECIMEN COLLECTION:

It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

PREPARATION:

Wright Stain, Modified, is supplied ready to use, although Wright stain may be diluted if placed on an automated instrument. See instructions which follow. Prepare Working Phosphate Buffer by diluting contents of one vial Phosphate Buffer pH 7.2 at 25°C to 3.8 liters or 1 gallon with water. Mix well to dissolve. Methanol is ready to use.

PRECAUTIONS:

Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet and product labeling for any updated risk, hazard or safety information.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:

Wright Stain, Modified, Catalog No. WS (WS16-500 ml; WS32-1L; WS80-2.5L; WS128-4L)

METHANOL, ACETONE FREE, Catalog No. M1775-1GA

RINSE SOLUTION 2, Catalog No. RS2-900 ml

Solutions

1. Place thoroughly dried blood film on an appropriate staining rack.
2. Place approximately 50 ml Wright Stain, Modified, in a Coplin jar.
3. After 30 seconds, without rinsing off Wright Stain from Step 2, add an equal volume of deionized water and mix thoroughly by gently blowing on slide.
4. Place thoroughly dried with deionized water and air dry.

Batch Staining With Hemastainer

1. Set timers to provide the following times for each station:
   - Station 1 - 30 seconds
   - Station 2 - 2 minutes
   - Station 5 - 3.5 minutes
   - Station 4 - 30 seconds
   - Station 5 - Skip and go to “air dry”

2. Prepare stations for staining in the following manner:
   - Station 1 - Absolute Methanol, 500 ml
   - Station 2 - Wright Stain, Modified 350 ml and Absolute Methanol 150 ml
   - Station 5 - Leave empty

3. Turn power switch to ON.
4. Set Auto/Manual switch to MANUAL.
5. Set Right/Left switch to LEFT.
6. Set swing switch to ON.
7. Set pump switch to AUTO.
8. Load basket with thoroughly air-dried blood films.
9. Avoid basket to hanger and tighten.
10. Start process by setting Auto/Manual switch to AUTO.

Batch Staining With Fisher Stainmaster Set program as follows:

<table>
<thead>
<tr>
<th>Event</th>
<th>Station</th>
<th>Reagent</th>
<th>Time (Minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Absolute Methanol</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Wright Stain, Modified</td>
<td>1.5</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Working Phosphate Buffer</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Deionized water</td>
<td>0.3</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Rinse Solution 2</td>
<td>0.7</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>Deionized water</td>
<td>0.3</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>Dry</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Batch Staining With Midas® II Set program as follows:

<table>
<thead>
<tr>
<th>Step</th>
<th>Bath</th>
<th>Reagent</th>
<th>Time (Seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Absolute Methanol</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Wright Stain, Modified</td>
<td>60-90</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Working Phosphate Buffer</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Running deionized water</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Dry</td>
<td>3 minutes or until dry</td>
</tr>
</tbody>
</table>

Unused baths may be omitted.

PERFORMANCE CHARACTERISTICS

Nuclei will be varying shades of purple. Cytoplasmic staining will be varying shades of blue to light pink. Fine reddish to lilac granules may be present in cytoplasm of some cell types. Basophilic will demonstrate dark blue black granules in the cytoplasm. Eosinophilic will demonstrate bright orange granules in the cytoplasm. Red blood cells should be pink to orange.

If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

REFERENCES


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Procedures W 031-08 Revisions: 2013-02 Revised: 2014-09