ThinPrep® Stain
User’s Manual
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Introduction

This User’s Manual is intended for customers using Hologic products to stain ThinPrep® Pap Test slides for cytologic screening.

The ThinPrep Stain User’s Manual provides our customers with standardized fixation and staining protocols using Hologic products in order to achieve consistent, high quality results. Technical information on reagents and protocols, basic troubleshooting techniques and Maintenance Schedules are included.

The ThinPrep Stain User’s Manual is written in a format to make it convenient for our customers to incorporate this information into their individual Laboratory Procedure Manuals.

WARNINGS/PRECAUTIONS

For proper handling, storage and disposal of each chemical, refer to the recommendations on the manufacturer’s Safety Data Sheet (SDS).

As with all laboratory procedures, universal precautions should be followed.
The Papanicolaou Stain (Pap Stain)

PRINCIPLE
Papanicolaou staining is the universal method for staining gynecologic slides for cytologic diagnosis. When performed properly, it results in:

- Crisp nuclear detail
- Differential cytoplasmic staining
- Transparent cells

The ThinPrep® Stain is used in conjunction with the ThinPrep Stain protocol and recommended reagents. The ThinPrep reagents and protocols have been developed by Hologic to produce consistent and standardized, high quality results for cytologic screening.

SPECIMEN REQUIREMENT
ThinPrep Pap Test slides processed on all approved ThinPrep Processors.
Fixation

**PRINCIPLE**

Fixatives are applied to specimens to preserve cytologic morphology. Immediate and proper fixation is critical to accurate cytologic diagnosis.

**FIXATION PROCEDURE**

Slides prepared on the ThinPrep® 3000 Processor are automatically treated with fixative (CellFyx™ Solution) in the instrument. No further fixation is required.

All other slides are deposited into a bath containing standard laboratory alcohol fixative (defined as 95% ethyl or reagent alcohol). For slides not intended for use with the ThinPrep Imaging System, certain laboratory procedures may require alternate fixatives in the bath or for it to remain empty.

For slides which must be shipped prior to staining, CellFyx Solution fixative must be applied. Refer to the CellFyx Solution Instructions for Use for details of applying CellFyx Solution to slides prior to shipping. Contact Hologic Customer Service for ordering.

**CAUTION:** No other fixative has been validated for transport.

For slides fixed in alcohol and intended for use with the ThinPrep Imaging System:

- Note that the alcohol in the fixative bath should be changed after 100 slides or daily, whichever comes first.
- Slides should be placed in a multi-slide holder (staining rack) immersed in a bath of 95% reagent alcohol or 95% ethyl alcohol.
- The slides must remain in the alcohol for at least 10 minutes prior to staining.
- Do not allow the slides to air-dry at any time prior to staining.
- Stainer bath solutions should be kept covered when not in use to minimize evaporation.
Staining

For storage temperatures and “opened bottle” limits of stain solutions, refer to the product label or its Instructions for Use.

**Note:** Filter ThinPrep® Nuclear Stain before initial use and when topping up, per standard laboratory practice.

**CAUTION:** For slides intended for use with the ThinPrep Imaging System, be sure to follow the staining instructions and applicable protocols exactly as stated. Do not substitute any solutions or stains required in the protocols, as this may compromise the performance of the ThinPrep Imaging System.

**REQUIRED MATERIALS FOR STAINING**

**WARNING**
Poisonous Substances
Flammable Liquids

Refer to SDS section of this manual.

*From Hologic*
ThinPrep Nuclear Stain
ThinPrep Rinse Solution or ThinPrep Rinse II Solution
ThinPrep Bluing Solution  ThinPrep Bluing II Solution
ThinPrep Orange G Solution
ThinPrep EA Solution

Follow local regulations or the manufacturer’s recommendations for handling and cleanup of spills.
Refer to manufacturer’s SDS for more information.

*From Other Suppliers*
Alcohols -

- 50% reagent or ethyl alcohol
- 70% reagent or ethyl alcohol
- 95% reagent or ethyl alcohol
- 100% reagent or ethyl alcohol
Clearing Agents -

- Xylene
- Shandon Xylene Substitute (Thermo Fisher Scientific)
- Pro-Par Clearant (Anatech, Ltd.)
- Histo-Clear® HS-200 (National Diagnostics)
- Histolene JJ031 (Fronine/Lomb Scientific) Australia/New Zealand

Water Quality
Distilled or deionized water that meets these Hologic specifications for Instrument Feed Water (CLSI, C3-A4 standard, 2006):

- ≤ 1.0 microSiemens/cm conductivity
- or ≥ 1.0 megOhm-cm resistivity

REAGENTS

ThinPrep Nuclear Stain
The ThinPrep Nuclear Stain is intended to be used in a Papanicolaou staining procedure in conjunction with the ThinPrep Rinse Solution or ThinPrep Rinse II Solution, ThinPrep Bluing Solution or ThinPrep Bluing II Solution, ThinPrep Orange G Solution, and ThinPrep EA Solution. The ThinPrep Nuclear Stain is an aqueous solution that serves to stain the nuclei of cells on cytologic slide preparations while maintaining cytoplasmic morphology.

ThinPrep Rinse Solution
The ThinPrep Rinse Solution is intended to be used in a Papanicolaou staining procedure in conjunction with the ThinPrep Nuclear Stain, ThinPrep Bluing Solution, ThinPrep Orange G Solution, and ThinPrep EA Solution. The ThinPrep Rinse Solution is an aqueous solution that removes excess ThinPrep Nuclear Stain from cellular components prior to bluing in cytologic slide preparations, enhancing nuclear staining and clarifying cytoplasms.

ThinPrep Rinse II Solution
The ThinPrep Rinse II Solution is intended to be used in a Papanicolaou staining procedure in conjunction with the ThinPrep Nuclear Stain, ThinPrep Bluing II Solution, ThinPrep Orange G Solution, and ThinPrep EA Solution. The ThinPrep Rinse II Solution is an alcohol-based solution that removes excess ThinPrep Nuclear Stain from cellular components on slide preparations for cytologic evaluation, enhancing nuclear staining and clarifying cytoplasms.
Staining

ThinPrep Bluing Solution

The ThinPrep Bluing Solution is intended to be used in a Papanicolaou staining procedure in conjunction with the ThinPrep Rinse Solution, ThinPrep Nuclear Stain, ThinPrep Orange G Solution, and ThinPrep EA Solution. The ThinPrep Bluing Solution is an aqueous, alkaline solution that causes cellular components stained with ThinPrep Nuclear Stain to darken to blue/violet, enhancing nuclear morphology in cytologic slide preparations.

ThinPrep Bluing II Solution

The ThinPrep Bluing II Solution is intended to be used in a Papanicolaou staining procedure in conjunction with the ThinPrep Rinse II Solution, ThinPrep Nuclear Stain, ThinPrep Orange G Solution, and ThinPrep EA Solution. The ThinPrep Bluing II Solution is an aqueous, alkaline solution that darkens the cellular components stained with the ThinPrep Nuclear Stain to blue/violet, enhancing nuclear morphology in cytologic slide preparations.

ThinPrep Orange G Solution

The ThinPrep Orange G Solution is intended to be used in a Papanicolaou staining procedure in conjunction with the ThinPrep Nuclear Stain, ThinPrep Rinse Solution or ThinPrep Rinse II Solution, ThinPrep Bluing Solution or ThinPrep Bluing II Solution, and ThinPrep EA Solution. The ThinPrep Orange G Solution is an alcohol-based solution that serves to stain the cytoplasm of cells on cytologic slide preparations. It will stain keratin in the cytoplasm of cells while maintaining nuclear staining and nuclear morphology.

ThinPrep EA Solution

The ThinPrep EA Solution is intended to be used in a Papanicolaou staining procedure in conjunction with the ThinPrep Nuclear Stain, ThinPrep Rinse Solution or ThinPrep Rinse II Solution, ThinPrep Bluing Solution or ThinPrep Bluing II Solution, and ThinPrep Orange G Solution. The ThinPrep EA Solution is an alcohol-based solution that serves to stain the cytoplasm of cells on cytologic slide preparations.

Reagent alcohol or ethyl alcohol

Reagent alcohol (a blend of 90% ethyl, 4-6% methyl and 4-6% isopropyl alcohols) or ethyl alcohol may be used in the staining procedure. The 100% alcohol must be virgin, but 95% may be from approved recycling systems.

Note: For ThinPrep Imaging System applications, see the next section, RECYCLED SOLVENTS, for approved systems and guidelines.
**WARNING**
Poisonous Substance
Flammable Liquid

Refer to manufacturer’s SDS for more information.

**Clearing Agents**

**Xylene**

For ThinPrep applications or for slides intended for use with the ThinPrep Imaging System, the following xylene alternatives are approved:

- Shandon Xylene Substitute (Thermo Fisher Scientific)
- Pro-Par Clearant (Anatech, Ltd.)
- Histo-Clear HS-200 (National Diagnostics)
- Histolene JJ031 (Fronine/Lomb Scientific) Australia/New Zealand

**CAUTION:** ThinPrep Imaging System applications require the clearing agent to be matched to the coverslipping media. See Table 1.3 on page 1.18.

**RECYCLED SOLVENTS**

**Systems and Guidelines**

Alcohols recycled via the following systems are acceptable for use with the ThinPrep Imaging System:

**Filtration Systems—Alcohol**

Creative Waste Solutions, Inc. (Bench Top Alcohol Recycling System)
www.cwsincorp.com or tel 888-795-8300

SunCycle Technologies, Inc. (Gravity Recycling Alcohol Cartridge (GRAC))
www.suncycletech.com or tel 866-786-2925

**Distillation Systems—Alcohol and Xylene**

CBG Biotech™
www.cbgbiotech.com or tel 800-941-9484

B/R Instrument
www.brinstrument.com or tel 410-820-8800

**Recycled Alcohol Guidelines**

General Guidelines (applies to all alcohol recycling)

- Laboratories must follow manufacturer’s recommendations for alcohol recycling.
- Only Hologic-recommended alcohols should be used—reagent or ethyl alcohol.
• Laboratories are responsible for ensuring that the recycled alcohol concentration used in the ThinPrep staining protocol meets Hologic’s recommendation.

**Filtration Systems—Alcohol only**

- Periodically have the output product independently tested (i.e., annually, at a minimum) to confirm proper function of the recycling system. Contact the manufacturer regarding this service.
- Only the 95% and 100% alcohols from the ThinPrep Stain setup should be put through the filtration system.
- 95% and 100% alcohols combined on the filtration system will result in an output alcohol concentration that will need to be adjusted to 95% prior to use in the ThinPrep Stain setup.

**Distillation Systems—Alcohol**

- Periodically have the output product independently tested (i.e., annually, at a minimum) to confirm proper function of the recycling system. Contact the manufacturer regarding this service.
- Only alcohols from the ThinPrep Stain setups can be distilled together.
- Alcohols from *non-ThinPrep stain* setups must **not** be co-mingled for recycling with ThinPrep alcohols.
- **ONLY 70%, 95% & 100% Reagent Alcohol or Ethyl Alcohol may be combined for recycling.** *(Note: 50% alcohol must be discarded according to the laboratory’s hazardous chemical waste disposal guidelines. If included for recycling, its high water content will reduce the recovery volume and significantly lower the output alcohol concentration.)*

**Testing:**

Test the collected waste alcohol solution to ensure it is free of xylene prior to recycling. Otherwise, the quality of the output alcohol will be compromised.

**Testing for Xylene Contaminant in Alcohol**

1. Mix the contents of the alcohol collection container thoroughly.
2. Pour or pipette 20 mL into a clean, dry vessel (e.g., graduated cylinder)
3. Add 20 mL of tap water, cover, and invert several times to mix.
   a. If any cloudiness appears within the solution (occurs almost immediately), xylene is present.
      Then:
      DISCARD (do not recycle) all the container’s contents according to the laboratory’s guidelines for hazardous chemical waste disposal.
      Clean the container using fresh absolute alcohol and dry.
      Begin new alcohol collection.
   b. If no cloudiness is present, no xylene is present.
      Proceed to recycle the collected alcohol.
Test the output alcohol
1. For xylene contaminant (See method above).
2. Use a hydrometer to determine concentration.
3. Adjust with water or absolute alcohol to 95%, 70%, or 50% as needed.

Special note for CBG Biotech units which are used to recycle both alcohol and xylene:

- When alcohol recycling is to follow xylene recycling, assure the “System flush” is properly performed to prevent contaminating alcohol with residual xylene. Refer to the CBG Biotech “Installation and Operating Instructions” manual for details.
- After a “System flush,” test output from the first recycled alcohol run to ensure xylene is not present.

**Caution:** Failure to follow the recommendations for fractional distillation in this section may result in recycled product which is not acceptable for use with the ThinPrep Imaging System.

**Distillation Systems—Xylene**

- Observe each manufacturer’s setup, maintenance and operation recommendations.
- Ensure the recycled xylene quality is suitable for use in ThinPrep staining protocols. Contact the respective manufacturer for their recommended laboratory test procedure.
- Periodically have the output product independently tested (i.e. annually, at minimum) to confirm proper function of the recycling system. Contact the manufacturer regarding this service.
- Only xylenes from ThinPrep Stain protocols can be distilled together. Xylenes from non-ThinPrep stain setups must not be co-mingled for recycling with ThinPrep xylenes.

**Caution:** Failure to follow all recommendations for fractional distillation in this section may result in recycled product which is not acceptable for use with the ThinPrep Imaging System.
Procedures For Staining

PROCEDURE NOTES FOR AUTOMATED STAINING

The following information is to be used in conjunction with the applicable staining protocol.

**WARNING**

Poisonous Substances
Flammable Liquids

Refer to the SDS for more information.

Bath solution heights should completely cover the slides at full immersion. Add fresh reagent as needed to maintain bath heights. It is recommended that the final clearing agent bath be filled to a level above the cell spot but below the frosted area of the slide. This will reduce the amount of mounting media overspreading the label area after the coverslip is applied.

Staining baths should be covered when not in use to minimize evaporation of solutions and oxidative effects on the stains.

Change all solutions when the number of slides stained equals 1 slide for every 1 mL of bath volume, or once a week, whichever comes first. (For example if your bath volume is 450 mL, then change the bath after 450 slides, or once a week, whichever comes first.)

**Note:** A list of the staining protocols including stain station maps for automated stainers is located at the “Protocols” tab of this manual. The stainers listed are the ones that have been validated for use with the ThinPrep® Stain Solutions and ThinPrep Pap slides processed on a ThinPrep Processor.

**Recommended Stain Bath Washing Procedure**

1. After emptying all stain baths, separate the baths for the clearing agent (Xylene, ProPar, etc.) from the others.
2. Clean clearing agent baths by wiping out any debris with a paper towel and letting them air-dry in a hood. **Do not use water in these baths.**
3. Wash out all of the other baths with a brush and water.
4. For baths that do not come clean with a brush and water:
   a. Use a solution of 1% hydrochloric acid in 70% reagent or ethyl alcohol. This works particularly well for baths stained with Orange G and EA Solutions.
   b. If step 4a does not clean the stain baths containing nuclear stain and those immediately following the nuclear stain, use a 10% solution of bleach in water. **Do not exceed a 10% bleach solution.**
5. Rinse out each bath several times with water and follow with a final rinse using distilled water. **Extra care must be taken to thoroughly rinse containers washed using the procedure in 4b, since any residual bleach can adversely affect the quality of subsequent staining.**
6. Allow the baths to air-dry or wipe them dry with paper towels before filling them for the next use.
Staining Troubleshooting

The following table is intended to guide the laboratory through standard troubleshooting practices if they are experiencing issues with the stain quality of slides stained with the ThinPrep Stain Solutions.

**Table 1.1: Troubleshooting**

<table>
<thead>
<tr>
<th>Category</th>
<th>Facts</th>
<th>Areas to Investigate</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>Frequency, onset, duration</td>
<td>Is there a noticeable pattern in when the problems occur?</td>
</tr>
<tr>
<td></td>
<td>What percentage of your work is being</td>
<td></td>
</tr>
<tr>
<td></td>
<td>affected?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>When was the issue initially seen?</td>
<td>Does it happen when the stainer is changed?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Is there anyone new performing staining?</td>
</tr>
<tr>
<td>Imager information</td>
<td></td>
<td>Are you seeing Imager slide events?</td>
</tr>
<tr>
<td>Staining</td>
<td>Stains</td>
<td>Are any of your stain solutions expired?</td>
</tr>
<tr>
<td></td>
<td>Check Solution Lot numbers</td>
<td>Is your Nuclear Stain solution open for 60 days or less?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Did you filter your Nuclear Stain solution before use?</td>
</tr>
<tr>
<td>Stainer</td>
<td>Are you following required stainer/</td>
<td>Verify staining protocol. (Refer to the protocol.)</td>
</tr>
<tr>
<td></td>
<td>staining protocols?</td>
<td>Verify stain bath layout. (Refer to the protocol.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Verify solutions in bath are at the appropriate fill level.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Verify slides are fully submerged in the post-stain solution baths throughout the</td>
</tr>
<tr>
<td></td>
<td></td>
<td>process.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Are you within the maximum allowable throughput limits for the stain table?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Are you changing your post Nuclear Stain water per protocol?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Are you cleaning stainer baths per recommended procedure? (Refer to the &quot;Recommended Stain Bath Washing Procedure&quot; on page 1.10.)</td>
</tr>
</tbody>
</table>
Table 1.1: Troubleshooting

<table>
<thead>
<tr>
<th>Category</th>
<th>Facts</th>
<th>Areas to Investigate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staining</td>
<td>Other Solutions</td>
<td>Water quality /Source/ How often or last time the system was verified?</td>
</tr>
<tr>
<td></td>
<td>Alcohol</td>
<td>Do you make alcohols or buy alcohols?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If you make alcohols, did you check the concentration of alcohols before use?</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Are you using recycled alcohol and following manufacturer’s guidelines?</td>
</tr>
<tr>
<td></td>
<td>Clearing Agents and Mounting Medium</td>
<td>Are you using an approved mounting medium / clearing agent combination? (Refer to tables 2 and 3 of this section.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Are you following the slide handling / drying guidelines?</td>
</tr>
</tbody>
</table>

Sample Biology

Certain Imaging System slide events (for example, 6615 or 6630) may be associated with patient biology when slides have excessive amounts of inflammation, bacteria, certain lubricants or blood. Contact Hologic Technical Support for guidance if there is an increase in such slide events.

Commonly Asked Questions and Answers

1. Does Hologic have a recommended stainer bath washing procedure?
   Yes, please see page 1.10 of this manual for details.

2. Can a lab destain/re-stain and then image slides if an applications issue arises which would normally call for this process?
   Hologic does not support destaining and re-staining slides intended for imaging with the ThinPrep Imaging System.

3. Does Hologic support staining slides by hand that are intended for imaging?
   Hologic does not support hand staining slides that are intended for imaging.

4. Does Hologic have a Non-Gyn protocol which uses the ThinPrep stain solutions?
   The ThinPrep Stain may be used for Non-Gyn slides, however, Hologic does not provide a protocol for this.

5. Can I substitute non-Hologic stain solutions in stainers for slides that are intended for imaging?
Hologic does not recommend using other stain solutions for slides intended for imaging with the ThinPrep Imaging System.

6. Can a lab self-validate stainers, protocols and mounting medium/clearing agent combinations for slides intended for imaging with the ThinPrep Imaging System? Hologic does not recommend that; self-validating does not provide the level of quality management that good lab practices recommend. Hologic validates stainers, stain protocols and mounting medium/clearing agent combinations for slides intended for imaging with the ThinPrep Imaging System.
Coverslipping

PURPOSE
The mounting medium creates a permanent bond between the slide and the coverslip. It protects the cellular material from air-drying and shrinkage and acts as a seal to deter fading of the stain over time.

COVERSLIPPING REQUIREMENTS
It is recommended that the bath in which the slides are held prior to coverslipping has the clearing agent filled to a level above the cell spot but below the frosted area of the slide. This will reduce the amount of mounting media overspreading the label area after the coverslip is applied.

Various mounting media have been evaluated by Hologic for use with ThinPrep slides. These are found in the table on page 1.16.

Glass coverslips #1 thickness, 24 mm x 50 mm are recommended. Note that ThinPrep slides for use on the ThinPrep Imaging System require coverslips long enough to cover the area including the fiducial marks that are printed on the microscope slide.

Hologic-approved film or tape coverslip material for use with automated coverslipping instrumentation is also acceptable, with the same note for length required for ThinPrep plus Imaging slides.

Required Materials
Table 1.2 lists the mounting media, clearing agents, coverslips and combinations of these items that have been evaluated and approved for use with the ThinPrep Imaging System. Table 1.3 lists coverslipping films approved for use with the ThinPrep Imaging System.

Automated Glass Coverslipping
Refer to manufacturer’s instructions for use of automated coverslipping instruments.

Note: Refer to Table 1.4 for recommendations for setting up a Sakura Tissue-Tek® FILM™ coverslipper for use with a ThinPrep Imaging System.

Note: Refer to detailed instructions for use of coverslipping tape/film on page 1.18.
Manual Glass Coverslipping

1. Quickly drain excess clearing agent from the slide.
2. Immediately apply sufficient mounting medium to the slide to minimize cornflaking, air bubbles or retraction. Avoid excessive medium, which may ooze out from under the coverslip and run down the edges of the slide.
3. Apply the coverslip using standard laboratory practice.
4. Using a lint-free wipe moistened in clearing agent, wipe the edges of the coverslip to clear any residual mounting medium.
5. Allow slides to dry completely (a minimum of 16 hours at room temperature or in a slide oven set at 37°C ± 3°C for 3 to 12 hours) before placing in Imager slide cassettes. Refer to the table on page 1.18.

CAUTION: Make sure the coverslips are centrally placed and do not overhang any edges of the slide.

Glass Coverslip Drying Time

After coverslipping, slides must be allowed to adequately “dry” before being placed in the Thin Prep Imaging System. A slide can be considered “dry” when the medium has cured sufficiently so there is no movement of the coverslip in response to normal laboratory handling.

Table 1.3 illustrates drying times for slides placed in an oven set at 37°C ± 3°C after automatic coverslipping.

Note: Drying times will differ depending upon variables such as the type of mounting medium, quantity of medium used, mounting method, temperature, and humidity. Manually coverslipped slides may have drying times longer than those indicated in Table 1.3.

It is the responsibility of the laboratory to monitor and ensure that adequate, but not excessive, medium is applied to the slides and that they “dry” sufficiently prior to being placed on the ThinPrep Imaging System.

CAUTION: Failure to allow slides to dry sufficiently may compromise the performance of the Imager.
Table 1.2: ThinPrep Imaging System Mounting Media/Clearing Agent Validation Summaries

<table>
<thead>
<tr>
<th>Mounting Media</th>
<th>Mounting Media Solvent</th>
<th>Media Distributor or Manufacturer</th>
<th>Clearing Agent</th>
<th>Automatic Coverslip System</th>
<th>Minimum Dry Time @ 37°C ± 3°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue-Tek® Glas (6419)</td>
<td>Xylene</td>
<td>Sakura Finetek</td>
<td>Xylene</td>
<td>Check coverslipper mfg. recommendations</td>
<td>1 hour</td>
</tr>
<tr>
<td>Richard-Allan Mounting Medium</td>
<td>Toluene</td>
<td>Thermo Fisher Scientific</td>
<td>Shandon Xylene Substitute (Histosolve)</td>
<td>DO NOT USE with Tissue-Tek glass coverslippers. Check coverslipper mfg. recommendations</td>
<td>1 hour</td>
</tr>
<tr>
<td>Medium (4111) also available as ClearVue Mountant (4211)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CoverSafe® Mounting Medium</td>
<td>D-Limonene</td>
<td>American Master Tech Scientific.</td>
<td>Shandon Xylene Substitute (Histosolve)</td>
<td>Check coverslipper mfg. recommendations</td>
<td>1 hour</td>
</tr>
<tr>
<td>Pertex® also available as Leica CV Mount</td>
<td>Xylene</td>
<td>Medite, Leica Biosystems and various distributors (international)</td>
<td>Xylene</td>
<td>Check coverslipper mfg. recommendations</td>
<td>1 hour</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Permount®</td>
<td>Toluene</td>
<td>Thermo Fisher Scientific</td>
<td>Xylene</td>
<td>DO NOT USE with Tissue-Tek glass coverslippers. Check coverslipper mfg. recommendations</td>
<td>3 hours</td>
</tr>
<tr>
<td>Neutral Balsam (China)</td>
<td>Xylene</td>
<td>Sinopharm Chemical Reagent Co.</td>
<td>Xylene</td>
<td>Check coverslipper mfg. recommendations</td>
<td>90 minutes</td>
</tr>
</tbody>
</table>
### Table 1.2: ThinPrep Imaging System Mounting Media/Clearing Agent Validation Summaries

<table>
<thead>
<tr>
<th>DPX Mountant</th>
<th>Xylene</th>
<th>Electron Microscopy Sciences, Leica Biosystems (USA)</th>
<th>Xylene</th>
<th>Check coverslipper mfg. recommendations</th>
<th>1 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caution: NOT DEPEX</td>
<td></td>
<td>Merck and various distributors (international)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgipath Micromount</td>
<td>Xylene</td>
<td>Leica Biosystems (USA)</td>
<td>Xylene</td>
<td>Check coverslipper mfg. recommendations</td>
<td>1 hour</td>
</tr>
</tbody>
</table>
Use of Coverslipping Tape/Film

1. Follow manufacturer’s recommendations for proper storage and operating conditions of the coverslipping product.
2. Place coverslipped slides flat in cardboard slide folders for a five-minute dry time at room temperature. Slides are then ready to be labeled (if needed) and imaged.
3. Slides may be imaged up to twenty-eight (28) days after coverslipping.
4. If using the Sakura Tissue-Tek FILM Coverslipper:
   - Refer to Table 1.3 for setup guidelines
   - Remove staining racks from the instrument immediately after coverslipping in complete. Do not let them remain in the output carousel.
   - Remove excess xylene by blotting the base of the stain rack on a lint-free wipe or paper towel. Then stand the rack up so slides are vertical.
   - Promptly remove slides from the rack, one at a time, wiping excess xylene from the back of each slide with a lint-free wipe.
   - Place slides flat in slide tray for drying.

<table>
<thead>
<tr>
<th>Coverslip</th>
<th>Coverslip Solvent</th>
<th>Distributor or Manufacturer</th>
<th>Clearing Agent</th>
<th>Minimum Dry Time at Room Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue-Tek Coverslipping Film (4770)</td>
<td>Xylene activated</td>
<td>Sakura Finetek</td>
<td>Xylene</td>
<td>5 min</td>
</tr>
<tr>
<td>KP Tape (3020)</td>
<td>Xylene activated</td>
<td>Klinipath bv The Netherlands</td>
<td>Xylene</td>
<td>5 min</td>
</tr>
</tbody>
</table>
Coverslipping

Coverslip Review

If any of the following are noted prior to imaging of the slide, the slide should be re-coverslipped per standard laboratory practice:

- Air bubbles

**CAUTION:** Large or numerous air bubbles can obscure cellular material.

- Retraction of mounting medium
- Coverslip is not straight or overhangs the edge of the slide.

Reagents

Follow manufacturer’s recommendations for handling and cleaning up spills. Refer to manufacturer’s SDS for further information.

---

**Table 1.4: Sakura Tissue-Tek FILM Coverslipper Settings for Use with the ThinPrep Imaging System**

<table>
<thead>
<tr>
<th>Setting</th>
<th>Tissue-Tek FILM Automated Coverslipper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coverslip Length</td>
<td>45 mm</td>
</tr>
<tr>
<td>Xylene Dispensing (Range 1 - 5)</td>
<td>Setting #1</td>
</tr>
<tr>
<td>Xylene Holding Bath</td>
<td>Full Bath</td>
</tr>
<tr>
<td>Spring Tension Setting</td>
<td>Adjust to maximum tension</td>
</tr>
</tbody>
</table>

Slide Drying:
- Remove staining racks from instrument immediately after coverslipping is complete. Do not let them sit in the output carousel.
- Remove excess xylene - dab base of stain rack on Kimwipe® or paper towel and stand the rack up so the slides are vertical.
- Immediately remove slides from rack and wipe excess xylene from back if slide with Kimwipe. Slides are now ready to label (if needed).
Limitations of Fixation, Staining and Coverslipping

The ThinPrep® reagents and protocols have been developed by Hologic to produce consistent and standardized, high quality results for cytologic screening. Substitution of reagents and/or changes to the Hologic staining protocols may produce less-than-optimal staining results.

If protocols are not followed or reagents are substituted, stain quality may be compromised.

Inadequately fixed specimens will not stain well.
Quality Control for the Papanicolaou Stain

PURPOSE

Regular monitoring of stain characteristics to:

- Ensure consistent well-stained slides for cytodiagnosis
- Detect stain quality issues prior to screening
- Comply with regulatory and accrediting agencies’ Quality Control requirements

PROCEDURE

Microscopic review of stained slides based on your laboratory guidelines.

EXPECTED RESULTS

General Expected Results

- Well-stained cells that are transparent
- Distinct chromatin
- Good differential cytoplasmic staining
- Even staining throughout the slide
- Reproducible and consistent results

Standard for ThinPrep® Nuclear Stain

Nuclear stain blue to blue/black

Threads between nuclear segments are visible in flattened polymorphonuclear leukocytes (PMNs).

Standard for ThinPrep Orange G Solution

Pale orange to yellow tint in the cytoplasm of non-keratinizing superficial squamous cells

Bright orange in keratinized superficial cells.

Standard for ThinPrep EA Solution

Non-keratinizing superficial and intermediate cells vibrant pinks and greens.

Nuclei and edges of overlapping cells are visible through the cytoplasm.
PROCEDURE NOTES

- Record results of microscopic review.
- Record problems/discrepancies and the remedial action taken.
Bibliography


Commission on Laboratory Accreditation, Laboratory Accreditation Program, Cytopathology Checklist, 2001.


Regulations (Standards - 29 CFR), National Research Council Recommendations Concerning Chemical Hygiene in Laboratories (Non-Mandatory) - 1910.1450 App A.


Maintenance Schedules

The maintenance schedules on the following pages may be used for photocopy purposes.

- ThinPrep Stain® Maintenance
- ThinPrep Stain Quality Control Check
- ThinPrep Stain Solutions - Inventory
ThinPrep® Stain Maintenance

Bath Volume: ________________

<table>
<thead>
<tr>
<th>Date</th>
<th>First Accession Number</th>
<th>Last Accession Number</th>
<th>Total Number of Slides Stained</th>
<th>All Solutions Changed* (Place ✓ in column)</th>
<th>Changed by</th>
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<tbody>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*Change all solutions when the number of slides stained equals 1 slide for every 1 mL of bath volume. (For example if your bath volume is 750 mL, then change the bath after 750 slides.)
This page intentionally left blank.
ThinPrep® Stain Quality Control Check

Month/Year: __________________

<table>
<thead>
<tr>
<th>Date</th>
<th>Prep Tech</th>
<th>QC Tech</th>
<th>Slide Number</th>
<th>Nuclear Stain</th>
<th>Cytoplasmic Stain</th>
<th>Comments</th>
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<tr>
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<td></td>
<td>Satisfactory</td>
<td>Satisfactory</td>
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<tr>
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This page is for photocopy purposes.
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ThinPrep® Stain Solutions--- Inventory

<table>
<thead>
<tr>
<th>ThinPrep Nuclear Stain</th>
<th>ThinPrep Bluing</th>
<th>ThinPrep Orange G</th>
<th>ThinPrep EA Solution</th>
<th>ThinPrep Rinse Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date Opened/Opened By</td>
<td>Lot Number</td>
<td>Discard Date*</td>
<td>Date Opened/Opened By</td>
<td>Lot Number</td>
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<tr>
<td>Date Opened/Opened By</td>
<td>Lot Number</td>
<td>Date Opened/Opened By</td>
<td>Lot Number</td>
<td>Date Opened/Opened By</td>
</tr>
<tr>
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<td>Date Opened/Opened By</td>
<td>Lot Number</td>
<td>Date Opened/Opened By</td>
</tr>
<tr>
<td>Date Opened/Opened By</td>
<td>Lot Number</td>
<td>Date Opened/Opened By</td>
<td>Lot Number</td>
<td>Date Opened/Opened By</td>
</tr>
<tr>
<td>Date Opened/Opened By</td>
<td>Lot Number</td>
<td>Date Opened/Opened By</td>
<td>Lot Number</td>
<td>Date Opened/Opened By</td>
</tr>
</tbody>
</table>

* Discard Date is 60 days from date opened
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**Technical Support**
For questions about ThinPrep Stain issues and related application issues, representatives from Technical Support are available by phone 7:00 a.m. to 7:00 p.m. EST Monday through Friday at 1-800-442-9892 Option 6 (USA and Canada).

For Technical Support outside USA and Canada:

<table>
<thead>
<tr>
<th>Country</th>
<th>Country Code</th>
<th>Phone Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td>+852 3526 0718</td>
<td>Netherlands 0800 022 6782</td>
</tr>
<tr>
<td>Australia</td>
<td>+61 2 9888 8000</td>
<td>Norway 800 155 64</td>
</tr>
<tr>
<td>Austria</td>
<td>0800 291 919</td>
<td>Portugal 800 841 034</td>
</tr>
<tr>
<td>Belgium</td>
<td>0800 773 78</td>
<td>Spain 900 994 197</td>
</tr>
<tr>
<td>Denmark</td>
<td>8088 1378</td>
<td>South Africa 0800 980 731</td>
</tr>
<tr>
<td>Finland</td>
<td>0800 114 829</td>
<td>Sweden 020 797 943</td>
</tr>
<tr>
<td>France</td>
<td>0800 913 659</td>
<td>Switzerland 0800 298 921</td>
</tr>
<tr>
<td>Germany</td>
<td>0800 183 0227</td>
<td>UK 0800 032 3318</td>
</tr>
<tr>
<td>Ireland (Rep)</td>
<td>1 800 554 144</td>
<td>Rest of the world 0041.21.633.39.26</td>
</tr>
<tr>
<td>Italy</td>
<td>800 786 308</td>
<td>Intl Fax number 0041.21.633.39.10</td>
</tr>
</tbody>
</table>

**Protocol for Returned Goods**
For returns on warranty-covered ThinPrep Stain items, contact Technical Support.

**Reordering ThinPrep Stain Supply Items**

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>ThinPrep Nuclear Stain</td>
<td>One 4-liter bottle</td>
<td>70780-001</td>
</tr>
<tr>
<td>ThinPrep Rinse Solution</td>
<td>One 4-liter bottle</td>
<td>70779-001</td>
</tr>
<tr>
<td>ThinPrep Bluing Solution</td>
<td>One 4-liter bottle</td>
<td>70793-001</td>
</tr>
<tr>
<td>ThinPrep Orange G Solution</td>
<td>One 4-liter bottle</td>
<td>70781-001, 70781-002</td>
</tr>
<tr>
<td>ThinPrep EA Solution</td>
<td>One 4-liter bottle</td>
<td>70782-001, 70782-002</td>
</tr>
<tr>
<td>Stain Set</td>
<td>1 gallon bottle of each of the five stain solutions</td>
<td>70897-001, 70897-002</td>
</tr>
<tr>
<td>ThinPrep Rinse II Solution*</td>
<td>One 4-liter bottle</td>
<td>ASY-04875</td>
</tr>
<tr>
<td>ThinPrep Bluing II Solution*</td>
<td>One 4-liter bottle</td>
<td>ASY-04876</td>
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</table>
### ORDERING INFORMATION

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>ThinPrep Stain Set II*</td>
<td>One 4-liter bottle each of Nuclear Stain Solution, Rinse II Solution, Bluing II Solution, Orange G Solution and EA Solution</td>
<td>PRD-01617, PRD-03753</td>
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</table>

*Note, ThinPrep Rinse II and Bluing II Solutions are designed to work together and cannot be mixed with, or used in conjunction with, the original ThinPrep Rinse and Bluing Solutions.*

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Part Number</th>
</tr>
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<tbody>
<tr>
<td>Stain Protocol Sakura 2000 Continuous using 95% alcohol fixed slides</td>
<td>One protocol</td>
<td>AW-19453-001</td>
</tr>
<tr>
<td>Stain Protocol Sakura 2000 Continuous using CellFyx’d slides</td>
<td>One protocol</td>
<td>AW-19454-001</td>
</tr>
<tr>
<td>Stain Protocol Sakura Tissue-Tek Prisma using 95% alcohol fixed slides</td>
<td>One protocol</td>
<td>AW-19456-001</td>
</tr>
<tr>
<td>Stain Protocol Sakura Tissue-Tek Prisma using CellFyx’d slides</td>
<td>One protocol</td>
<td>AW-19457-001</td>
</tr>
<tr>
<td>Expansion Stain Protocol Sakura Tissue-Tek Prisma using 95% alcohol fixed slides</td>
<td>One protocol</td>
<td>AW-19462-001</td>
</tr>
<tr>
<td>Expansion Stain Protocol Sakura Tissue-Tek Prisma using CellFyx’d slides</td>
<td>One protocol</td>
<td>AW-19463-001</td>
</tr>
<tr>
<td>Stain Protocol Leica Autostainer XL using 95% alcohol fixed slides</td>
<td>One protocol</td>
<td>AW-19452-001</td>
</tr>
<tr>
<td>Stain Protocol Leica Autostainer XL using CellFyx’d slides</td>
<td>One protocol</td>
<td>AW-19451-001</td>
</tr>
<tr>
<td>Stain Protocol Leica ST5020 Multistainer using 95% alcohol fixed slides</td>
<td>One protocol</td>
<td>AW-19458-001</td>
</tr>
<tr>
<td>Stain Protocol Leica ST5020 Multistainer using CellFyx’d slides</td>
<td>One protocol</td>
<td>AW-19459-001</td>
</tr>
<tr>
<td>Stain Protocol Shandon Varistain Gemini using 95% alcohol fixed slides</td>
<td>One protocol</td>
<td>AW-19455-001</td>
</tr>
</tbody>
</table>
### Ordering Information

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stain Protocol Medite TST 44 using 95% alcohol fixed slides</td>
<td>One protocol</td>
<td>AW-19460-001</td>
</tr>
<tr>
<td>Stain Protocol Medite TST 44 using CellFyx’d slides</td>
<td>One protocol</td>
<td>AW-19461-001</td>
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<tr>
<td>Stain Protocol using Rinse II and Bluing II Solutions on the Leica Autostainer XL</td>
<td>One protocol</td>
<td>AW-19466-001</td>
</tr>
<tr>
<td>Stain Protocol using Rinse II and Bluing II Solutions on the Leica ST5020 Multistainer</td>
<td>One protocol</td>
<td>AW-19465-001</td>
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<tr>
<td>Stain Protocol using the Rinse II and Bluing II Solutions on the Sakura Tissue-Tek DRS 2000 Stainer</td>
<td>One protocol</td>
<td>AW-19467-001</td>
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<tr>
<td>Stain Protocol using the Rinse II and Bluing II Solutions on the Sakura Tissue-Tek Prisma Autostainer</td>
<td>One protocol</td>
<td>AW-19468-001</td>
</tr>
<tr>
<td>Stain Protocol using the Rinse II and Bluing II Solutions on the Sakura Tissue-Tek Prisma Stainer in Expansion Configuration</td>
<td>One protocol</td>
<td>AW-19469-001</td>
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<tr>
<td>Stain Protocol using the Rinse II and Bluing II Solutions on the Medite TST 44 Stainer</td>
<td>One protocol</td>
<td>AW-19470-001</td>
</tr>
<tr>
<td>Stain Protocol using the Rinse II and Bluing II Solutions on the Hologic Compass Stainer using 95% alcohol fixed slides</td>
<td>One protocol</td>
<td>AW-19471-001</td>
</tr>
</tbody>
</table>
Ordering Information

**Mailing Address**
Hologic, Inc.
250 Campus Drive
Marlborough, MA 01752 USA

**Remittance Address**
Hologic, Inc.
PO Box 3009
Boston, MA 02241-3009 USA

**Business Hours**
Hologic’s business hours are 8:30 a.m. to 5:30 p.m. EST Monday through Friday, excluding holidays.

**Customer Service**
Product orders are placed through Customer Service by phone during business hours at 1-800-442-9892 Option 5.

Orders can also be faxed to the attention of Customer Service at 1-800-409-7591.

A copy of Hologic’s limited warranty and other terms and conditions of sale may be obtained by contacting Customer Service at the numbers listed above.
Safety Data Sheets
Safety Data Sheets

ThinPrep® Nuclear Stain Solution
ThinPrep Rinse Solution
ThinPrep Bluing Solution
ThinPrep Orange G Solution
ThinPrep EA Solution
CellFyx™ Solution
ThinPrep Rinse II Solution
ThinPrep Bluing II Solution

The Safety Data Sheet (SDS) for each solution may be requested from Hologic Technical Support, or found on-line at www.hologicsds.com.
## Staining Protocols

The following staining protocols are available from Hologic:

<table>
<thead>
<tr>
<th>Stainer</th>
<th>Protocol Description</th>
<th>Protocol Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sakura Tissue-Tek DRS 2000 Slide Stainer</td>
<td>Stain Protocol Sakura 2000 Continuous using 95% alcohol fixed slides</td>
<td>AW-19453-001</td>
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<tr>
<td>Sakura Tissue-Tek Prisma Automated Slide Stainer</td>
<td>Stain Protocol Sakura Tissue-Tek Prisma using 95% alcohol fixed slides</td>
<td>AW-19456-001</td>
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<td>Sakura Tissue-Tek Prisma Automated Slide Stainer</td>
<td>Stain Protocol Sakura Tissue-Tek Prisma using CellFyx’d slides</td>
<td>AW-19457-001</td>
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<tr>
<td>Sakura Tissue-Tek Prisma Automated Slide Stainer</td>
<td>Expansion Stain Protocol Sakura Tissue-Tek Prisma using 95% alcohol fixed slides</td>
<td>AW-19462-001</td>
</tr>
<tr>
<td>Leica Autostainer XL</td>
<td>Stain Protocol Leica Autostainer XL using 95% alcohol fixed slides</td>
<td>AW-19452-001</td>
</tr>
<tr>
<td>Leica Autostainer XL</td>
<td>Stain Protocol Leica Autostainer XL using CellFyx’d slides</td>
<td>AW-19451-001</td>
</tr>
<tr>
<td>Leica ST5020 Multistainer</td>
<td>Stain Protocol Leica ST5020 Multistainer using 95% alcohol fixed slides</td>
<td>AW-19458-001</td>
</tr>
<tr>
<td>Leica ST5020 Multistainer</td>
<td>Stain Protocol Leica ST5020 Multistainer using CellFyx’d slides</td>
<td>AW-19459-001</td>
</tr>
<tr>
<td>Thermo Shandon Varistain Gemini Slide Stainer</td>
<td>Stain Protocol Shandon Varistain Gemini using 95% alcohol fixed slides</td>
<td>AW-19455-001</td>
</tr>
<tr>
<td>Stainer</td>
<td>Protocol Description</td>
<td>Protocol Number</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>--------------------------------------------------------------------------------------</td>
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<tr>
<td>Medite TST 44 Slide Stainer</td>
<td>Stain Protocol Medite TST 44 using 95% alcohol fixed slides</td>
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</tr>
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<td>AW-19466-001</td>
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<td>AW-19465-001</td>
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<td>Stain Protocol using the Rinse II and Bluing II Solutions on the Sakura Tissue-Tek Prisma Autostainer</td>
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<td>Sakura Tissue-Tek Prisma Automated Slide Stainer</td>
<td>Stain Protocol using the Rinse II and Bluing II Solutions on the Sakura Tissue-Tek Prisma Stainer in Expansion Configuration</td>
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<td>Hologic Compass Stainer</td>
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<td>AW-19471-001</td>
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