

ThinPrep® Stain Protocol Using Rinse II and Bluing II Solutions on the LEICA AUTOSTAINER XL

Use this protocol ONLY when ThinPrep Rinse II and Bluing II Solutions (P/Ns ASY-04875, ASY-04876) are components of the ThinPrep Stain.

Use this protocol for staining slides fixed with 95% alcohol.

Convert wash stations to reagent stations by installing wash plugs in Stations Wash 1 thru Wash 4 and replacing wash containers with reagent containers. Reagent containers are used in all stations.

Maintain bath heights to completely cover the slides at full immersion. (Note: Bath fill volume = 450 ml)

Caution: Change the Distilled / Instrument Feed Water bath at Station 5 after every **four racks** of slides.

Required Setup Parameters: Dips = 10; Move (Dip = 2, Up = 9, Down = 2)				
Step	Station	Solution	Time	Exact
---	LOAD	95% reagent or ethyl alcohol	N/A	N/A
1	1	70% reagent or ethyl alcohol	1 min	No
2	2	50% reagent or ethyl alcohol	1 min	No
3	3	Distilled or Instrument Feed Water (IFW) ¹	1 min	No
4	4	ThinPrep Nuclear Stain	7 min	Yes
5	5	Distilled or Instrument Feed Water ¹ (Replace after every 4 racks.)	10 sec	Yes
6	6	ThinPrep Rinse II Solution	1 min	Yes
7	7	Distilled or Instrument Feed Water ¹	30 sec	No
8	Wash_1	ThinPrep Bluing II Solution	30 sec	Yes
9	Wash_2	Distilled or Instrument Feed Water ¹	30 sec	No
10	Wash_3	Distilled or Instrument Feed Water ¹	30 sec	No
11	Wash_4	95% reagent or ethyl alcohol	30 sec	No
12	Wash_5	ThinPrep Orange G Solution	2 min	Yes
13	8	95% reagent or ethyl alcohol	15 sec	Yes
14	9	95% reagent or ethyl alcohol	15 sec	Yes
15	10	ThinPrep EA Solution	4 min	Yes
16	11	95% reagent or ethyl alcohol	1 min	Yes
17	12	95% reagent or ethyl alcohol	1 min	Yes
18	13	100% reagent or ethyl alcohol	30 sec	No
19	14	100% reagent or ethyl alcohol	30 sec	No
20	15	100% reagent or ethyl alcohol	30 sec	No
21	16	Xylene or other approved Hologic clearing agent ²	1 min	No
22	17	Xylene or other approved Hologic clearing agent ²	3 min	No
Optional 23	18	Xylene or other approved Hologic clearing agent ²	1 min	No
---	EXIT	Xylene or other approved Hologic clearing agent ²	N/A	N/A
Remove slides to a separate clearing bath then coverslip with the appropriate Hologic approved mountant. ²				

¹ Hologic specification for IFW: ≥ 1.0 megohm-cm Resistivity **OR** ≤ 1.0 μ Siemens/cm Conductivity (Refer to Clinical and Laboratory Standards Institute (CLSI) document C3-A4, 2006)

² See ThinPrep Stain User's Manual, Section 1, "STAINING" or contact Hologic for current list of Hologic approved clearing agents and mounting media.



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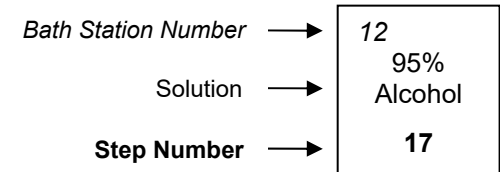
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Solution Stations

Leica Autostainer XL

Using ThinPrep Rinse II and Bluing II Solutions

For slides fixed with 95% alcohol



<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>	<i>7</i>	<i>Wash 1</i>	<i>Wash 2</i>	<i>Wash 3</i>	<i>Wash 4</i>	<i>Wash 5</i>	<i>Oven</i>
70% Alcohol	50% Alcohol	Distilled Water	Nuclear Stain	Distilled Water	Rinse II Solution	Distilled Water	Bluing II Solution	Distilled Water	Distilled Water	95% Alcohol	OG Solution	
1	2	3	4	5	6	7	8	9	10	11	12	
	<i>18</i>	<i>17</i>	<i>16</i>	<i>15</i>	<i>14</i>	<i>13</i>	<i>12</i>	<i>11</i>	<i>10</i>	<i>9</i>	<i>8</i>	
Xylene	Open (<i>Optional Xylene</i>)	Xylene	Xylene	100% Alcohol	100% Alcohol	100% Alcohol	95% Alcohol	95% Alcohol	EA Solution	95% Alcohol	95% Alcohol	95% Alcohol
EXIT	23	22	21	20	19	18	17	16	15	14	13	LOAD