

ThinPrep® Stain Protocol for the LEICA AUTOSTAINER XL using 95% alcohol fixed slides

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.

Caution: Change the Distilled / Instrument Feed Water bath at Station 5 after every **four racks** of slides. Maintain bath heights to completely cover the slides at full immersion. (Note: Bath fill volume = 450 ml.)

Required Setup Parameters: Dips = 10; Move (Dip = 2, Up = 9, Down = 2)					
Step	Solution		Time	Exact	
LOAD	95% reagent or ethyl alcohol		N/A	N/A	
1	70% reagent or ethyl alcohol		1 min	No	
2	50% reagent or ethyl alcohol		1 min	No	
3	Distilled or Instrument Feed Water (IFW) ¹		1 min	No	
4	ThinPrep Nuclear Stain		7 min	Yes	
5	Distilled or Instrument Feed Water ¹ (Replace after every 4 racks.)		10 sec	Yes	
6	ThinPrep Rinse Solution		1 min	Yes	
7	Distilled or Instrument Feed Water ¹		30 sec	No	
8	ThinPrep Bluing Solution		30 sec	Yes	
9	Distilled or Instrument Feed Water ¹		30 sec	No	
10	50% reagent or ethyl alcohol		30 sec	No	
11	95% reagent or ethyl alcohol		30 sec	No	
12	ThinPrep Orange G Solution		2 min	Yes	
13	95% reagent or ethyl alcohol		15 sec	Yes	
14	95% reagent or ethyl alcohol		15 sec	Yes	
15	ThinPrep EA Solution		4 min	Yes	
16	95% reagent or ethyl alcohol		1 min	Yes	
17	95% reagent or ethyl alcohol		1 min	Yes	
18	100% reagent or ethyl alcohol		30 sec	No	
19	100% reagent or ethyl alcohol		30 sec	No	
20	100% reagent or ethyl alcohol		30 sec	No	
21	Xylene or other Hologic approved clearing agent ²		1 min	No	
L1	L2		Layout 1	Layout 2	
22	22	Xylene or other approved Hologic clearing agent ²	3 min	1 min	No
---	23	Xylene or other approved Hologic clearing agent ²	n/a	2 min	No
23	24	Xylene or other approved Hologic clearing agent ²	EXIT	EXIT	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 medias.



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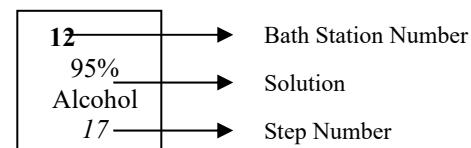


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Solution Stations – Layouts 1 and 2

Leica Autostainer XL

For staining slides fixed with 95% alcohol



Layout 1

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

Front row includes three xylene baths.

Layout 2

1 70% Alcohol <i>1</i>	2 50% Alcohol <i>2</i>	3 Distilled Water <i>3</i>	4 Nuclear Stain <i>4</i>	5 Distilled Water <i>5</i>	6 Rinse Solution <i>6</i>	7 Distilled Water <i>7</i>	Wash 1 Bluing Solution <i>8</i>	Wash 2 Distilled Water <i>9</i>	Wash 3 50% Alcohol <i>10</i>	Wash 4 95% Alcohol <i>11</i>	Wash 5 OG Solution <i>12</i>	Oven
Exit Xylene <i>24</i>	18 Xylene <i>23</i>	17 Xylene <i>22</i>	16 Xylene <i>21</i>	15 100% Alcohol <i>20</i>	14 100% Alcohol <i>19</i>	13 100% Alcohol <i>18</i>	12 95% Alcohol <i>17</i>	11 95% Alcohol <i>16</i>	10 EA Solution <i>15</i>	9 95% Alcohol <i>14</i>	8 95% Alcohol <i>13</i>	Load 95% Alcohol

Front row includes four xylene baths.