## ThinPrep<sup>®</sup> Stain Protocol using Rinse II and Bluing II Solutions on the Sakura Tissue-Tek Prisma<sup>®</sup> and Prisma<sup>®</sup> Plus Autostainers

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

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

<u>Caution</u>: Change the Distilled / Instrument Feed Water at Step 5 after every **NINE** racks of slides. Maintain bath heights to completely cover the slides at full immersion. (Note: Full bath volume is 820 ml.)

<i>Required Setup Parameters:</i> Mix (Amplitude = 30 mm, Frequency = 10, Speed = 1), Basket Lifting Speed = 1								
Step	Solution Name	Time	Delay	Mix				
1	Start Station (Reagent or Ethyl alcohol: 70%)	::						
2	Reagent or Ethyl alcohol: 50%	0:01:00	**	on				
3	Distilled or Instrument Feed Water (IFW) <sup>1</sup>	0:01:00	**	on				
4	ThinPrep Nuclear Stain	0:07:00	==	on				
5	Distilled or Instrument Feed Water <sup>1</sup> (Replace after every <b>9</b> racks.)	0:00:10	==	on				
6	ThinPrep Rinse II Solution	0:01:00	==	on				
7	Distilled or Instrument Feed Water <sup>1</sup>	0:00:30	==	on				
8	ThinPrep Bluing II Solution	0:00:30	==	on				
9	Distilled or Instrument Feed Water <sup>1</sup>	0:00:30	**	on				
10	Distilled or Instrument Feed Water <sup>1</sup>	0:00:30	**	on				
11	Reagent or Ethyl alcohol: 95%	0:00:30	**	on				
12	ThinPrep Orange G Solution	0:02:00	==	on				
13	Reagent or Ethyl alcohol: 95%	0:00:15	==	on				
14	Reagent or Ethyl alcohol: 95%	0:00:15	==	on				
15	ThinPrep EA Solution	0:04:00	==	on				
16	Reagent or Ethyl alcohol: 95%	0:01:00	==	on				
17	Reagent or Ethyl alcohol: 95%	0:01:00	==	on				
18	Reagent or Ethyl alcohol: 100%	0:00:30	**	on				
19	Reagent or Ethyl alcohol: 100%	0:00:30	**	on				
20	Reagent or Ethyl alcohol: 100%	0:00:30	**	on				
21	Xylene or other Hologic approved clearing agent <sup>2</sup>	0:01:00	**	on				
22	Xylene or other Hologic approved clearing agent <sup>2</sup>	0:03:00	**	on				
23	End Station (Xylene or other Hologic approved clearing agent <sup>2</sup> )	::						
Ren	nove slides to a separate clearing bath then coverslip with the appropri	ate Hologic-ap	proved mou	untant. <sup>2</sup>				

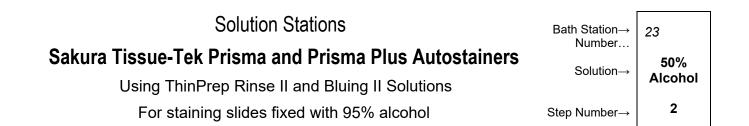
\*\* Unlimited, == Exact (no delay)

<sup>1</sup> Hologic specification for IFW: ≥1.0 megohm-cm Resistivity <u>OR</u> ≤1.0 µSiemens/cm Conductivity (Refer to Clinical and Laboratory Standards Institute (CLSI) document C3-A4, 2006)

<sup>2</sup> 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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1	2	3	4	5	6	7	8
95% Alcohol	95% Alcohol	ThinPrep EA	95% Alcohol	95% Alcohol	ThinPrep Orange G	95% Alcohol	DRY 1
17	16	15	14	13	12	11	
9	10	11	12	13	14	15	16
100% Alcohol	100% Alcohol	100% Alcohol	Distilled Water	ThinPrep Bluing II	Distilled Water	Distilled Water	DRY 2
18	19	20	7	8	9	10	
17	18	19	20	21	22	23	24
	Xylene	Xylene	ThinPrep Rinse II	ThinPrep Nuclear	Distilled Water	50% Alcohol	
	22	21	6	4	3	2	
	E1	E2/SN	E3/SN	S3/SN	S2/SN	S1	
(Link to cover- slipper)	Xylene			Distilled Water	70% Alcohol	70% Alcohol	
	23			5	1	1	
E# = End Station SN = Solution Station S# = Start Station							-

\* Configuration shown is for Prisma Model 6130 or Prisma Plus Model 6170. In Models 6131 and 6171, Station 24 is replaced with two heat stations.