ThinPrep® Stain Protocol
using Rinse II and Bluing II Solutions on the
Sakura Tissue-Tek Prisma® and Prisma® 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.

Caution: 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.)

Required Setup Parameters: 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)[1]	0:01:00	**	on
4	**ThinPrep Nuclear Stain**	0:07:00	==	on
5	Distilled or Instrument Feed Water[1] (Replace after every **9** racks.)	0:00:10	==	on
6	**ThinPrep Rinse II Solution**	0:01:00	==	on
7	Distilled or Instrument Feed Water[1]	0:00:30	==	on
8	**ThinPrep Bluing II Solution**	0:00:30	==	on
9	Distilled or Instrument Feed Water[1]	0:00:30	**	on
10	Distilled or Instrument Feed Water[1]	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 [2]	0:01:00	**	on
22	Xylene or other Hologic approved clearing agent [2]	0:03:00	**	on
23	End Station (Xylene or other Hologic approved clearing agent [2])	--:--:--	------	------
Remove slides to a separate clearing bath then coverslip with the appropriate Hologic-approved mountant. [2]				

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

[1] Hologic specification for IFW: ≥1.0 megohm-cm Resistivity OR ≤1.0 µSiemens/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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Solution Stations

Sakura Tissue-Tek Prisma and Prisma Plus Autostainers

Using ThinPrep Rinse II and Bluing II Solutions

For staining slides fixed with 95% alcohol

Bath Station→ Number…	*23*
Solution→	**50% Alcohol**
Step Number→	**2**

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