

ThinPrep® Stain Protocol for the Sakura Tissue-Tek® Prisma® and Prisma® Plus Autostainers using 95% alcohol fixed slides

Use 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 Solution	0:01:00	==	on
7	Distilled or Instrument Feed Water ¹	0:00:30	==	on
8	ThinPrep Bluing Solution	0:00:30	==	on
9	Distilled or Instrument Feed Water ¹	0:00:30	**	on
10	Reagent or Ethyl alcohol: 50%	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 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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Solution Stations

Sakura Tissue-Tek Prisma and Prisma Plus Autostainers

For staining slides fixed with 95% alcohol.

Bath Station→	23
Number...	50% Alcohol
Solution→	
Step...	
Number→	2

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	Distilled Water	50% Alcohol	DRY 2
18	19	20	7	8	9	10	
17	18	19	20	21	22	23	24 *
	Xylene	Xylene	ThinPrep Rinse	ThinPrep Nuclear	Distilled Water	50% Alcohol	
	22	21	6	4	3	2	
(Link to Cover- slipper)	<i>E1</i>	<i>E2/SN</i>	<i>E3/SN</i>	<i>S3/SN</i>	<i>S2/SN</i>	<i>S1</i>	
	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.