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

Use for staining slides with Small Solution Reservoirs (Sakura P/N 6145).

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

Caution: Change the Distilled / Instrument Feed water bath at Step 5 (Station 48) after every **NINE** racks of slides. Maintain bath heights to completely cover the slides at full immersion. (Note: Bath fill volume is 280 ml, except for Stations 47 through 52 which are standard, 820 ml baths.)

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 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 medias.

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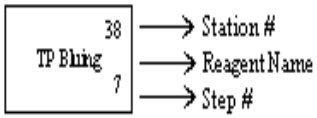
Expansion Solution Stations
Sakura Tissue-Tek Prisma and Prisma Plus Autostainers

For staining slides fixed with 95% alcohol



1 **THINPREP ORANGE G** **12**	*3* 95% ALCOHOL **11**	*5* 50% ALCOHOL **10**	*7 (W4)*	*8 (W3)*	*9 (W2)*	*10 (W1)*	*13 (D1a)*
2 95% ALCOHOL **13**	*4* 95% ALCOHOL **14**	*6* DISTILLED WATER **9**	*11*	*12*	*54*	*55*	*14 (D1b)*
15 95% ALCOHOL **16**	*17* **THINPREP EA** **15**	*19* **THINPREP BLUING** **8**	*21*	*23*	*25*	*27*	*29 (D2a)*
16 95% ALCOHOL **17**	*18* 100% ALCOHOL **18**	*20* DISTILLED WATER **7**	*22*	*24*	*26*	*28*	*30 (D2b)*
31 100% ALCOHOL **20**	*33* 100% ALCOHOL **19**	*35* **THINPREP RINSE** **6**	*37*	*39*	41	43	*45**
32 XYLENE **21**	34 XYLENE **22**	*36* **THINPREP NUCLEAR STAIN** **4**	*38*	*40*	*42*	*44*	*46**
53 LINK	*47 (E1)* XYLENE **23 / End**	*48 (E2)* POST-NUCLEAR DISTILLED WATER **5**	*49 (E3)* DISTILLED WATER **3**	*50 (S3)* 50% ALCOHOL **2**	*51 (S2)* 70% ALCOHOL **1 / Start**	*52 (S1)* 70% ALCOHOL **1 / Start**	

E# = Exit Station W# = Running Water S# = Start Station D# = Drying Station

* Configuration shown is for Prisma Model 6130 or Prisma Plus Model 6170. In Models 6131 and 6171, Stations 45 & 46 are replaced with heat stations.