

ThinPrep® Expansion 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.

Use this protocol for staining slides using Small Solution Reservoirs, 250 ml (Sakura P/N 6145).

Use 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 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 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 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 media.



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Expansion 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	6
Solution →	DISTILLED WATER
Step Number →	9

1 TP OG SOLUTION 12	3 95% ALCOHOL 11	5 DISTILLED WATER 10	7 (W1)	8 (W2)	9 (W3)	10 (W4)	13 (D1)
2 95% ALCOHOL 13	4 95% ALCOHOL 14	6 DISTILLED WATER 9	11	12	54	55	14 (D2)
15 95% ALCOHOL 16	17 TP EA SOLUTION 15	19 TP BLUING II SOLUTION 8	21	23	25	27	29 (D3)
16 95% ALCOHOL 17	18 100% ALCOHOL 18	20 DISTILLED WATER 7	22	24	26	28	30 (D2)
31 100% ALCOHOL 20	33 100% ALCOHOL 19	35 TP RINSE II SOLUTION 6	37	39	41	43	45*
32 XYLENE 21	34 XYLENE 22	36 TP NUCLEAR STAIN 4	38	40	42	44	46*
LINK to Coverslipper	47 (E1) XYLENE 23	48 (E2/SN) POST-TP NUCLEAR DISTILLED WATER 5	49 (E3/SN) DISTILLED WATER 3	50 (S3/SN) 50% ALCOHOL 2	51 (S2) 70% ALCOHOL 1	52 (S1) 70% ALCOHOL 1	

E# - Exit Station

SN – Solution

W# - Running Water

S# - Start Station

D# - Dry Station

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