ThinPrep® Stain Protocol using Rinse II and Bluing II Solutions on the SAKURA TISSUE-TEK DRS™ 2000 Autostainer

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 fixed with 95% alcohol.

At the Start Process screen, be sure "Mode" (F3) is set to "Continuous".

Remove all wash nozzles and insert plugs. Use only Solution Reservoirs in all stations.

Maintain bath heights to completely cover the slides at full immersion. (Note: Bath fill volume = 650 ml)

Caution: Change Distilled / Instrument Feed Water bath at Station 9 after every **eight racks** of slides.

NOTE: *Stations 21 and 22 must first be assigned as Wash stations prior to assigning other reagent stations.*

Required Setup Parameters (System Setup Screen):
Mix Amplitude = **30mm**, Mix Frequency = **10**, Mix Speed = **1,** Basket Lifting Speed = **1**

Step	Station	Solution	Duration	Time	Mix
1	S (27)	Start (95% reagent or ethyl alcohol)	–	––' ––"	
	S (26)	Start (95% reagent or ethyl alcohol)	–	––' ––"	
2	25	70% reagent or ethyl alcohol	Infinity	01' 00"	+
3	24	50% reagent or ethyl alcohol	Infinity	01' 00"	+
4	23	Distilled or Instrument Feed Water (IFW) [1]	Infinity	01' 00"	+
5	8	**ThinPrep Nuclear Stain**	Exact	07' 00"	+
6	9	Distilled or Instrument Feed Water[1] (Change after **8** racks.)	Exact	00' 10"	+
7	10	**ThinPrep Rinse II Solution**	Exact	01' 00"	+
8	11	Distilled or Instrument Feed Water[1]	Infinity	00' 30"	+
9	12	**ThinPrep Bluing II Solution**	Exact	00' 30"	+
10	13	Distilled or Instrument Feed Water[1]	Infinity	00' 30"	+
11	1	Distilled or Instrument Feed Water[1]	Infinity	00' 30"	+
12	2	95% reagent or ethyl alcohol	Infinity	00' 30"	+
13	3	**ThinPrep Orange G Solution**	Exact	02' 00"	+
14	4	95% reagent or ethyl alcohol	Exact	00' 15"	+
15	5	95% reagent or ethyl alcohol	Exact	00' 15"	+
16	6	**ThinPrep EA Solution**	Exact	04' 00"	+
17	7	95% reagent or ethyl alcohol	Exact	01' 00"	+
18	20	95% reagent or ethyl alcohol	Exact	01' 00"	+
19	19	100% reagent or ethyl alcohol	Infinity	00' 30"	+
20	18	100% reagent or ethyl alcohol	Infinity	00' 30"	+
21	17	100% reagent or ethyl alcohol	Infinity	00' 30"	+
22	16	Xylene or other Hologic approved clearing agent[2]	Infinity	01' 00"	+
23	15	Xylene or other Hologic approved clearing agent[2]	Infinity	03' 00"	+
24	E (14)	End (Xylene)	Infinity	––' ––"	+
Remove slides to a separate clearing bath then coverslip with the appropriate Hologic approved mountant.[2]					

[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.

EC REP

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Solution Stations

Sakura Tissue-Tek DRS™ 2000 Autostainer

Using ThinPrep Rinse II and Bluing II Solutions

For slides fixed with 95% alcohol

Bath Station Number →	*25*
Solution →	70% Alcohol
Step Number →	**2**

Upper Level

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
Distilled Water	95% Alcohol	**OG Solution**	95% Alcohol	95% Alcohol	**EA Solution**	95% Alcohol	**Nuclear Stain**	Distilled Water	**Rinse II Solution**	Distilled Water	**Bluing II Solution**	Distilled Water
11	**12**	**13**	**14**	**15**	**16**	**17**	**5**	**6**	**7**	**8**	**9**	**10**

14	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*DRY*
Xylene **END**	Xylene	Xylene	100% Alcohol	100% Alcohol	100% Alcohol	95% Alcohol			Distilled Water	50% Alcohol	70% Alcohol	95% Alcohol **START**	95% Alcohol **START**	
24	**23**	**22**	**21**	**20**	**19**	**18**			**4**	**3**	**2**	**1**	**1**	

Lower Level