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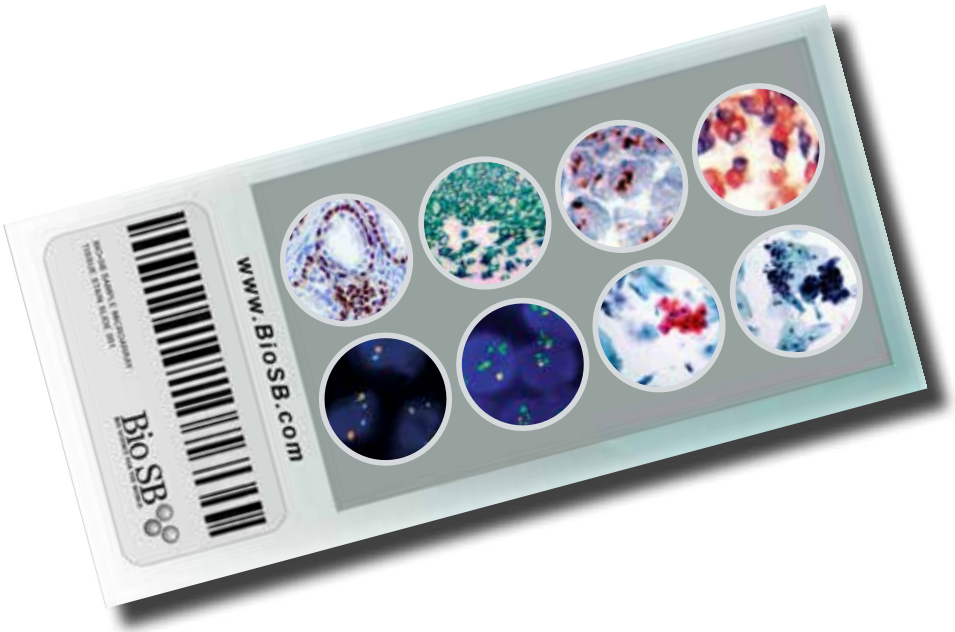

Bio SB TintoDetector ImmunoDNA System

Compact System for Molecular Pathology

Instruction Manual

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V3 08/01/18

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TintoDetector Manual Table of Contents

Overview

The Bio SB TintoDetector system is a capillary gap based system which can be used for Immunohistochemistry (IHC), Immunocytochemistry (ICC), ImmunoFluorescence (IF), Fluorescent in-situ hybridization (FISH), and Chromogenic in-situ hybridization (CISH) applications. The TintoDetector is an open system, and reagents from any supplier can be used.

The Bio SB TintoDetector is comprised of several components, which can be found in the diagram below (Figure 1.1). The components of the TintoDetector include...

1. TintoDetector System (Qty: 1)_____BSB 7000
2. TintoDetector Incubator (Qty: 1)_____BSB 7002
3. TintoDetector 30-Well Reagent Holder (Qty: 5)_____BSB 7004
4. TintoDetector Absorbent Pads (Qty: 10)_____BSB 7036
5. TintoDetector Slide Holder (Qty: 1, See Figure 1.2)_____BSB 7003
6. TintoDetector Cap Gap Plus Slides (Box of 72)_____BSB 7006
7. Plastic Staining Dish (Qty: 8) _____BSB 7009

Figure 1.1

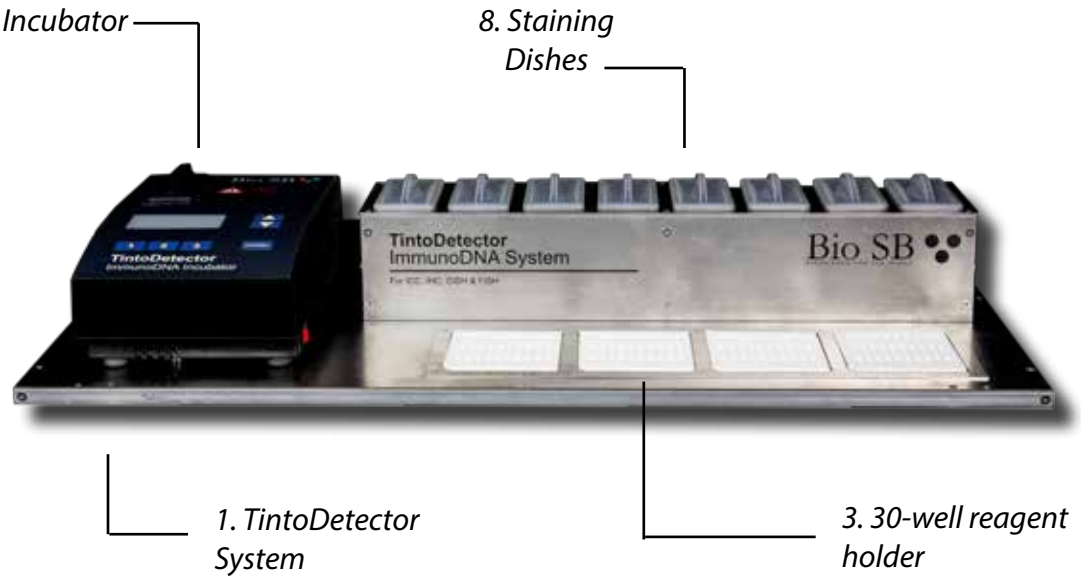
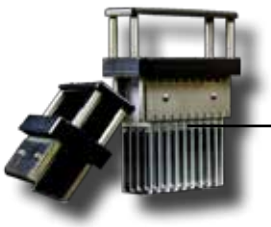


Figure 1.2



TintoDetector Components

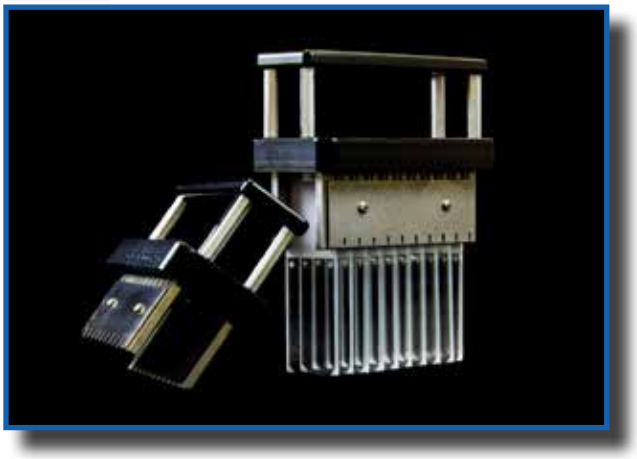
Incubator

See Figure 2.1

The TintoDetector Incubator is capable of reaching temperatures up to 110°C, and is capable of storing 3 temperature presets. The incubator is used to apply varying temperatures typically used in CISH and FISH protocols that involve denaturing of nucleic acids, probe hybridization, and other steps related to CISH/FISH implementation.

Figure 2.1

Figure 2.2

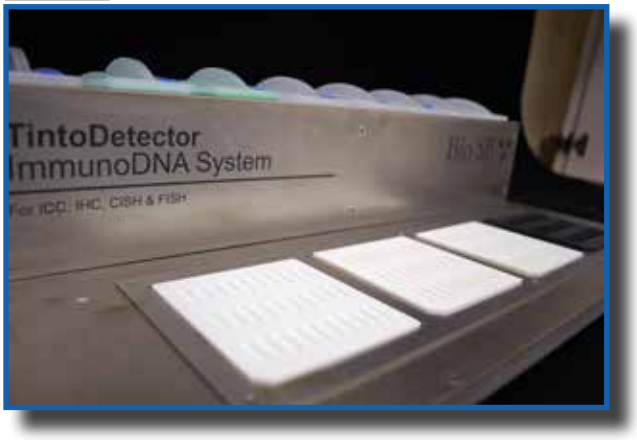


TintoDetector Slide Holder

See Figure 2.2

The TintoDetector Slide Holder is an extremely durable capillary gap slide holder that is capable of holding 20 capillary gap slides, and easily fits into the TintoDetector Incubator. The TintoDetector Slide holder can be used for all IHC, ICC, IF, CISH, and FISH applications.

Figure 2.3



TintoDetector 30 Well Reagent Holder

See Figure 2.3

The TintoDetector 30-Well Reagent Holder allows for the application of up to 200 micro-liters of reagent to a paired set of slides. The 30-well reagent holders can be used to apply any reagent used in IHC, ICC, IF, CISH and FISH protocols.

TintoDetector Components (Continued)

Figure 2.4

Staining Dish Rack
See Figure 2.4

All TintoDetector staining dishes are capable of holding xylene and alcohol as well as any washes, buffers, or special stains for use in IHC, ICC, IF, CISH, and FISH protocols. All staining dishes are capable of holding 200 mL of reagent.

TintoDetector Specifications

TintoDetector Dimensions	36in. x 16in. x 11in. (91cm. x 41cm. x 28cm.)
TintoDetector Weight	31 pounds (14kg)
TintoDetector Incubator Voltage Requirements	110 Volt / 220 Volt
Supported Protocols	IHC, ICC, IF, CISH and FISH

Guidelines

Ensure that TintoDetector is placed in a well ventilated area, as the built in incubator must have access to free air-flow to ensure proper operating temperatures are reached. When operating incubator, ensure that humidity chamber (located on back left of unit) is filled with up to 10 mL of distilled water.

Ensure unit is placed on a level surface.

Always use proper safety guidelines when working with toxic and flammable reagents in your laboratory. All protocols listed within the TintoDetector manual are guidelines only, and are meant as a sample application of the TintoDetector. Always reference the supplier protocol before using the TintoDetector.

Installation Checklist

Upon receiving your TintoDetector system, ensure that all necessary parts are included:

- ☐ TintoDetector Base Station (1)
- ☐ TintoDetector Incubator (1)
- ☐ TintoDetector Slide Holder (1)
- ☐ TintoDetector 30-Well Reagent Holder (4)
- ☐ TintoDetector Staining Dishes and Lids(8)
- ☐ TintoDetector Absorbent Pads (10)
- ☐ Slides (1 box of 72)

Installation Procedure

- ☐ Place base station on level surface.
- ☐ Place 30-Well Reagent Holders on base station.
- ☐ Place staining dishes in base station.
- ☐ Plug incubator into electrical outlet.
- ☐ Turn incubator on using power switch on base of unit.
- ☐ Fill staining dishes with 200 mL reagent.
- ☐ Set incubator presets to desired temperatures:
  1. Turn on incubator using switch on base of unit
  2. Fill Humidity chamber with 10mL distilled H_20.
  3. Press 1, 2, or 3 Temperature Preset button.
  4. Press Up/Down arrow to adjust temperature setting for preset.
  5. To recall a preset, simply press the 1, 2, or 3 Preset button.

Figure 3.1

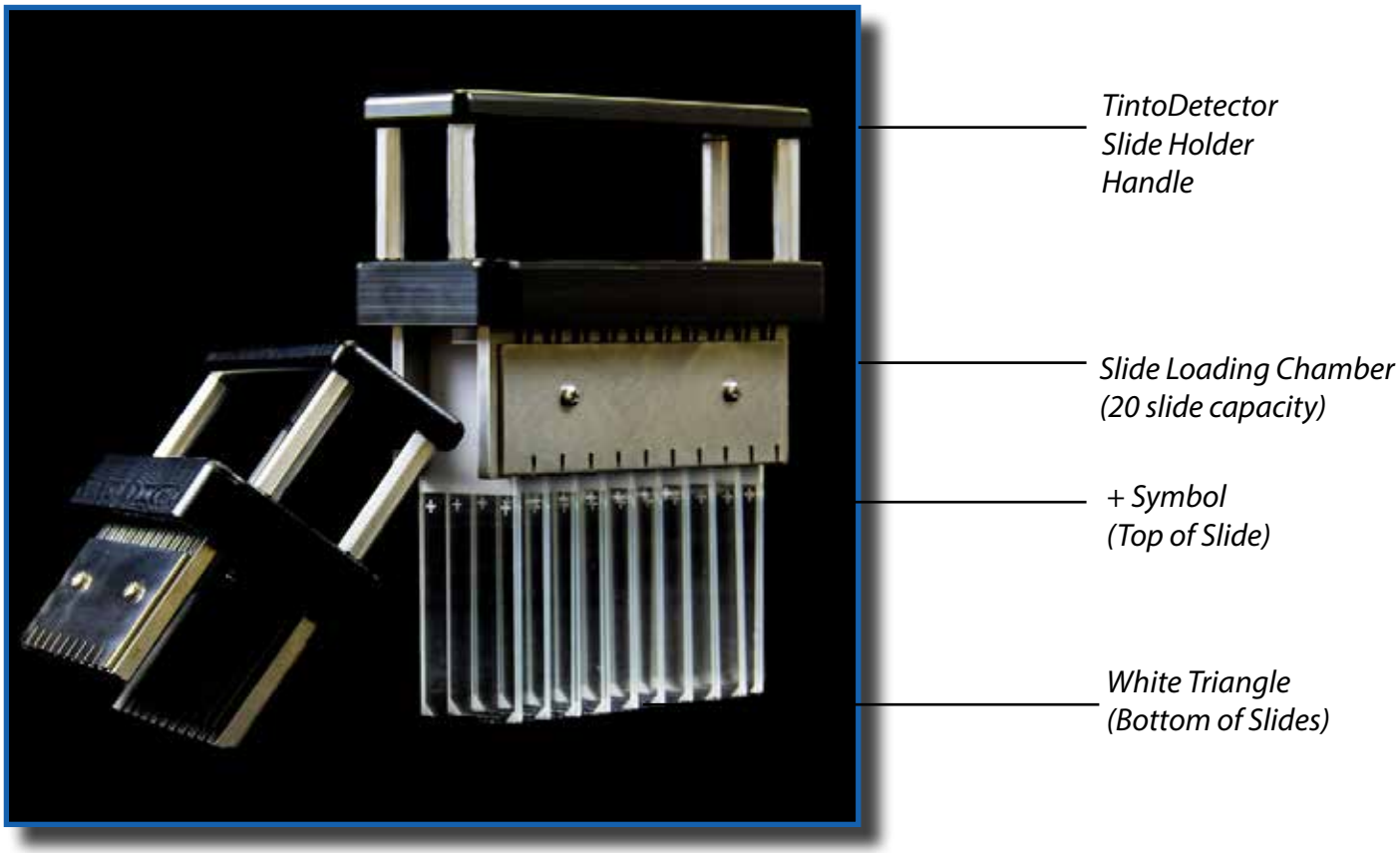
TintoDetector Slide Holder

See Figure 3.2

The TintoDetector System uses a specially designed capillary gap slide with raised white triangles at the bottom of the slide (see figure 3.2). When using the TintoDetector Slide Holder, ensure that the following procedures are followed...

- Slides are paired face to face.
- If a single slide or odd number must be used, pair with a blank slide.
- Insert slides so that portion of slide with white triangle faces downward when unit is held.
- Ensure that TintoDetector Cap Gap Slides or TintoDetector Cap Gap Plus Slides are used.
- Ensure all bottom edges of slides are aligned to ensure proper capillary gap action.
- Up to 20 capillary gap slides can be used in one TintoDetector Slide Holder.

Figure 3.2

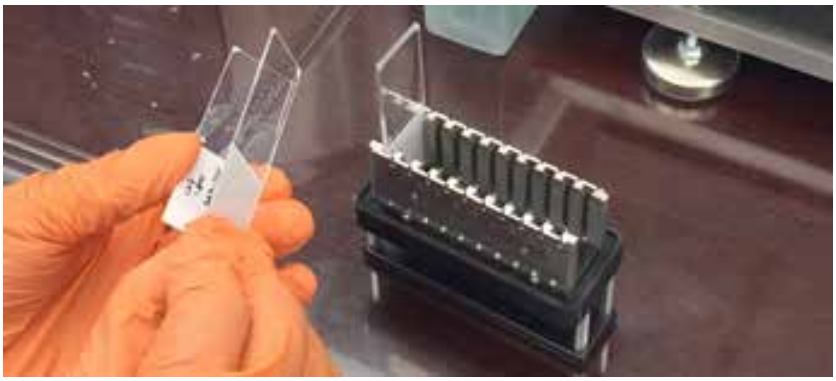


TintoDetector Wash Procedure

The TintoDetector Slide Holder, TintoDetector Cap Gap Plus Slides, 30-Well Reagent Holder and TintoDetector Absorbent Pads are used in unison to draw reagent as well as perform stringency washes.

Step 1 - Load Slides

Load slides in TintoDetector Holder, face to face and properly ordered.



Step 2 - Apply Reagents to Reagent Holder

Apply reagent to TintoDetector 30-well Reagent Holder. Each reagent well can hold about 200 micro-liters of reagent.

Step 3 - Draw Reagents into Slides

Place TintoDetector slide holder over 30-well reagent holder, ensuring that reagents line up with slides. Press slide holder against reagent holder. Capillary gap action will draw reagent. Transfer TintoDetector Slide Holder to Incubator.

Step 4 - Incubate

Incubate Slide Holder using the TintoDetector Incubator

Step 5 - Rinse

After the reagent incubation, eliminate the used reagent into an absorbent pad, then draw washing buffer into the capillary space and repeat the washing process 3 to 5 times. After washing proceed to draw the next step reagent before another incubation.

Additional Information

If you would like additional resources on how your TintoDetector system can be used, feel free to visit the following sites:

YouTube

- Our You Tube channel contains up to date information about your TintoDetector as well as video tutorials on how your TintoDetector can be used with different reagents and protocols.
- Channel URL: http://www.biosb.com/technical-resources/videos/

Web

- Our website strives to have the most up to date information for your needs. Additionally our website lists IHC, ICC, IF, FISH and CISH reagents that can be used with your TintoDetector system.
- Channel URL: http://www.biosb.com

Support

- Email: info@biosb.com
- Phone (International): +1-805-692-2768
- Phone (United States): 1-800-561-1145
- Bio SB online trouble-shooting form: http://www.biosb.com/product-troubleshootingcomplaint-form/
- Reordering: info@biosb.com

International Distribution

- Our most up to date list of distributors is available at www.biosb.com/distributors

TintoDetector Accessories

All the components below are compatible with the TintoDetector system.

TintoRetriever Pressure Cooker

BSB 7008

The TintoRetriever Pressure Cooker with built in temperature gauge allows for quick and efficient epitope and nucleic acid retrieval for all IHC, ICC, IF, CISH and FISH protocols.

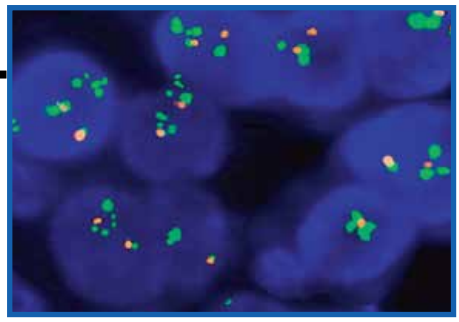
Bio SB IHC Detection Systems
Bio SB TintoAntibodies

All Bio SB IHC ancillaries, chromogens, counter-stains, biotin & polymer detection systems are compatible with the TintoDetector. Additionally all Bio SB TintoAntibodies are compatible and ready-to-use with the TintoDetector.

CISH/FISH Probes and Kits

www.biosb.com/fish-cish

All CISH and FISH probes, ancillaries and reagents are compatible with the TintoDetector. If you would like to see a list of CISH and FISH Probes distributed by Bio SB visit our ISH site at www.biosb.com/fish-cish



Bio SB Automated TintoStainer

BSB 7034

Need an automated IHC system to complement your TintoDetector? The Bio TintoStainer is capable of automated IHC/ICC and uses digital bar code printing and scanning to ensure protocol accuracy and fast turnaround time.

TintoDetector
Sample Applications

IHC, ICC, CISH and FISH sample TintoDetector Setup and Protocols

The following pages highlight some protocols for use in IHC, ICC, CISH and FISH applications. The protocols on the following pages are meant to serve as guidelines which highlight the flexibility and openess that the TintoDetector offer end-users.

Always refer to the manufacturers supplied technical procedures whenever performing any IHC, ICC, CISH or FISH protocol.

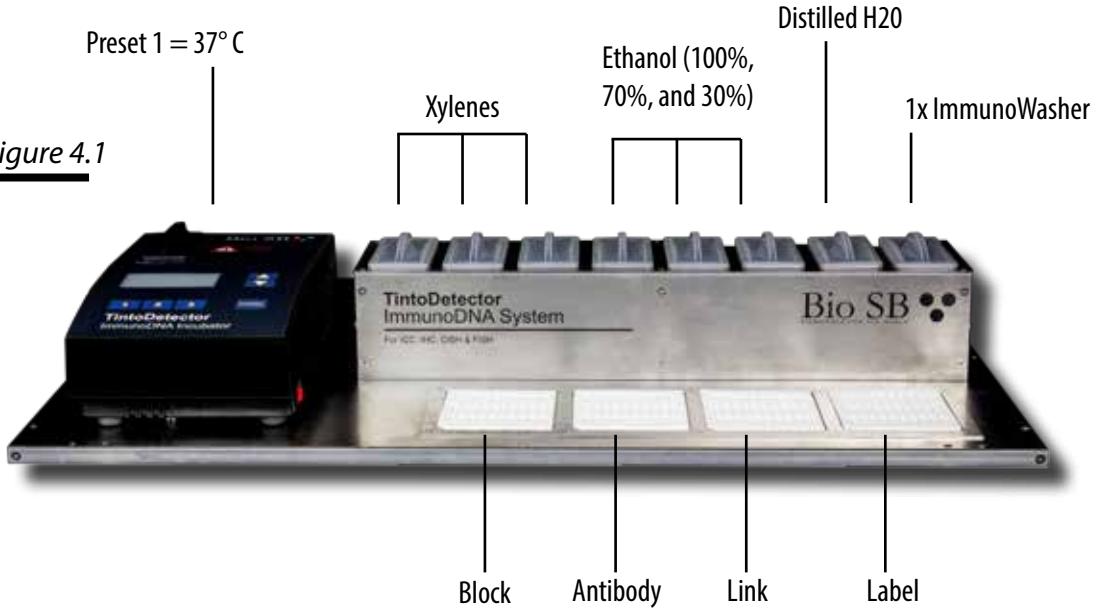
Sample Applications

As previously mentioned, The TintoDetector system can be used for IHC, ICC, IF, CISH, and FISH. Included are some sample protocols to give the end user an idea of how the TintoDetector can be used in a variety of applications.

Immunohistochemistry (Biotin Detection System) TintoDetector Setup

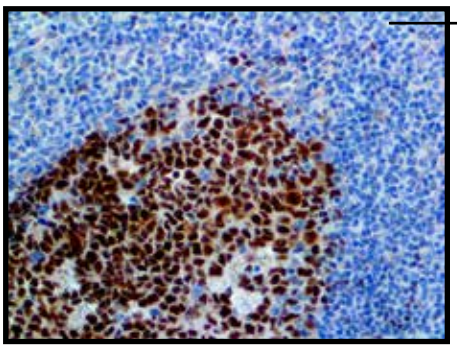
Bio SB Mouse/Rabbit ImmunoDetector DAB HRP Brown Detection System
Catalog Number: BSB 0005

Figure 4.1



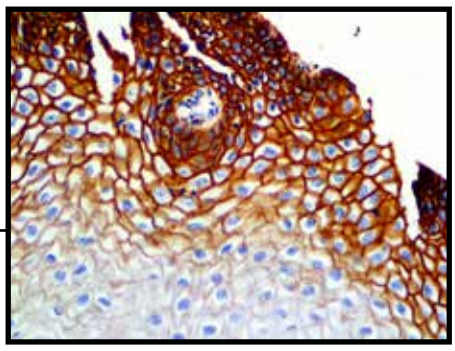
Sample Stains

Figure 4.2



BCL-6
Clone: RBT-bcl6
BSB 5080

Figure 4.3



EGFR
Clone: 31G7
BSB 5472

Sample IHC ImmunoDetector DAB HRP Brown Detection System Protocol

Bio SB Mouse/Rabbit ImmunoDetector DAB HRP Brown Detection System
Catalog Number: BSB 0005

Note: If no temperature specified, assume room temperature.

	Step	Solution	Time/Temp (°C)
☐	Deparaffinize	Xylenes	3 x 3′
☐	Dehydrate	Alcohols	3 x 3′
☐	Hydrate	Distilled Water	1 x 5″
☐	Wash	ImmunoWasher	1 x 2′
☐	Heat Pretreatment	HIER Solution	1 x 15′ in Pressure Cooker
☐	Cool	HIER Solution	1 x 20′
☐	Pair Capillary Gap Slides	Not Applicable	Not Applicable
☐	Wash	ImmunoWasher	5 x 10″
☐	Peroxidase Block	Peroxidase Block	1 x 5′ @ 37°C
☐	Wash	ImmunoWasher	5 x 10″
☐	Primary Antibody	Primary Antibody	1 x 30-60′. @ 37°C
☐	Wash	ImmunoWasher	5 x 10″
☐	Biotin Link	Biotin Link	1 x 10′ @ 37°C
☐	Wash	ImmunoWasher	5 x 10″
☐	HRP Label	HRP Label	1 x 10′ @ 37°C
☐	Wash	ImmunoWasher	5 x 10″
☐	DAB*	DAB Chromogen*	1 x 5-10′ @ 37°C
☐	Wash	ImmunoWasher	5 x 10″
☐	Counter-stain	Hematoxylin	1 x 30″
☐	Wash	ImmunoWasher	5 x 10″
☐	Dehydrate	Alcohol	3 x 2′
☐	Prepare for Mount	Xylene	3 x 2′
☐	Mount	PermaMounter	Not Applicable

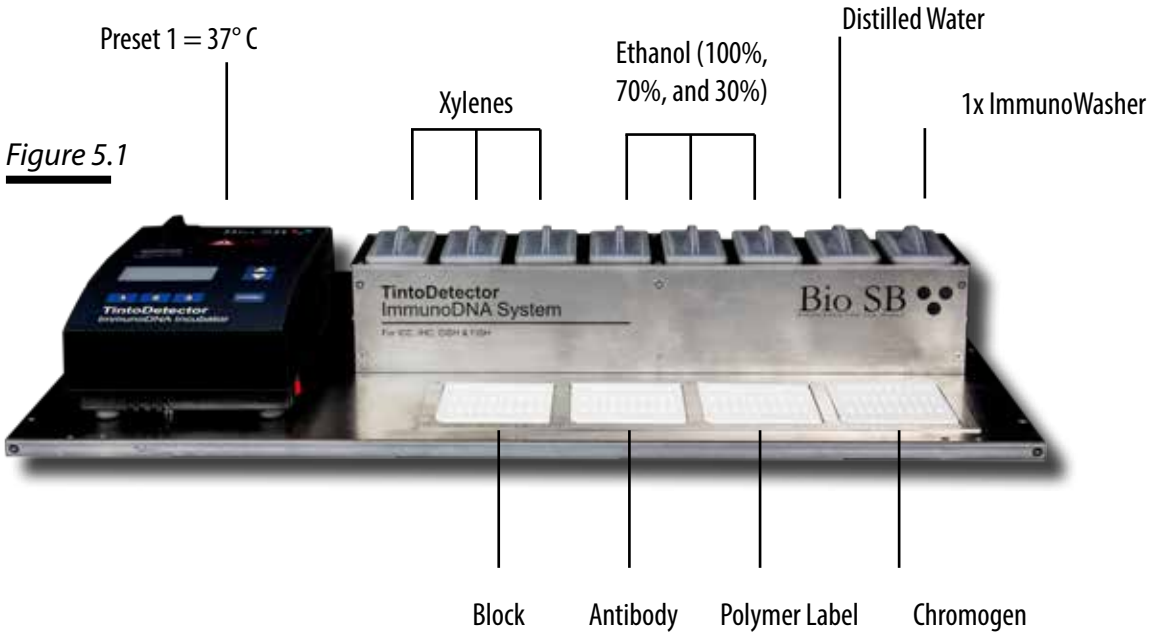
** DAB Chromogen Preparation = 1mL of Substrate : 1 mL of Chromogen. Mix and use.*

Note: Incubation times, antibody, chromogen, and kit components will vary by vendor, application, and use. Remember to read all instructions that come with detection kit to ensure accurate implementation of protocol. The protocol provided above is used solely to demonstrate the TintoDetector's ability to handle IHC.

Immunohistochemistry (Polymer-Based Detection System) TintoDetector Setup

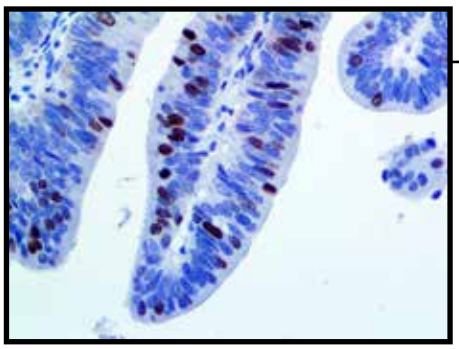
Bio SB Mouse/Rabbit PolyDetector DAB HRP Brown Detection System
Catalog Number: BSB 0205

Figure 5.1



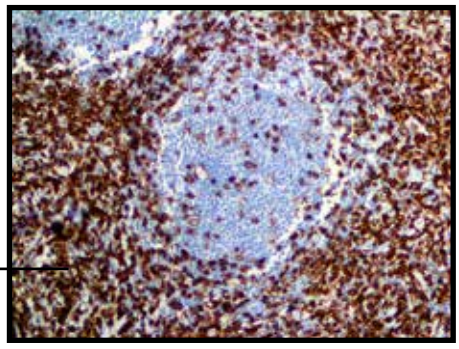
Sample Stains

Figure 5.2



Survivin
Clone: EP119
BSB 2225

Figure 5.3



CD43
Clone: MT1
BSB 5241

Sample IHC PolyDetector DAB HRP Brown Detection System Protocol

Bio SB Mouse/Rabbit PolyDetector DAB HRP Brown Detection System
Catalog Number: BSB 0205

Note: If no temperature specified, assume room temperature.

	Step	Solution	Time/Temp (°C)
☐	Deparaffinize	Xylenes	3 x 3′
☐	Dehydrate	Alcohols	3 x 3′
☐	Hydrate	Distilled Water	1 x 5″
☐	Wash	ImmunoWasher	1 x 2′
☐	Heat Pretreatment	HIER Solution	1 x 15′ in Pressure Cooker
☐	Cool	HIER Solution	1 x 20′
☐	Pair Capillary Gap Slides	Not Applicable	Not Applicable
☐	Wash	ImmunoWasher	5 x 10″
☐	Peroxidase Block	Peroxidase Block	1 x 5′ @ 37°C
☐	Wash	ImmunoWasher	5 x 10″
☐	Primary Antibody	Primary Antibody	1 x 45-60′ @ 37°C
☐	Wash	ImmunoWasher	5 x 10″
☐	HRP Label (Polymer)	HRP Label (Polymer)	1 x 45′ @ 37°C
☐	Wash	ImmunoWasher	5 x 10″
☐	DAB*	DAB Chromogen*	1 x 5-10′ @ 37°C
☐	Wash	ImmunoWasher	5 x 10″
☐	Counter-stain	Hematoxylin	1 x 30″
☐	Wash	ImmunoWasher	5 x 10″
☐	Dehydrate	Alcohol	3 x 2′
☐	Prepare for Mount	Xylene	3 x 2′
☐	Mount	PermaMounter	Not Applicable

** DAB Chromogen Preparation = 1mL of Substrate : 1 mL of Chromogen. Mix and use.*

Note: Incubation times, antibody, chromogens, and kit components will vary by vendor, application, and use. Remember to read all instructions that come with detection kit to ensure accurate implementation of protocol. The protocol provided above is used solely to demonstrate the TintoDetector's ability to handle IHC.

Sample IHC PolyDetector Plus DAB HRP Brown Detection System Protocol

Bio SB Mouse/Rabbit PolyDetector Plus DAB HRP Brown Detection System
Catalog Number: BSB 0261

Note: If no temperature specified, assume room temperature.

	Step	Solution	Time/Temp (°C)
☐	Deparaffinize	Xylenes	3 x 3′
☐	Dehydrate	Alcohols	3 x 3′
☐	Hydrate	Distilled Water	1 x 5″
☐	Wash	ImmunoWasher	1 x 2′
☐	Heat Pretreatment	HIER Solution	1 x 15′ in Pressure Cooker
☐	Cool	HIER Solution	1 x 20′
☐	Pair Capillary Gap Slides	Not Applicable	Not Applicable
☐	Wash	ImmunoWasher	5 x 10″
☐	Peroxidase Block	Peroxidase Block	1 x 5′ @ 37°C
☐	Wash	ImmunoWasher	5 x 10″
☐	Primary Antibody	Primary Antibody	1 x 30-60′ @ 37°C
☐	Wash	ImmunoWasher	5 x 10″
☐	Link	Mouse/Rabbit Link	1 x 15′ @ 37°C
☐	Wash	ImmunoWasher	5 x 10″
☐	HRP Label (Polymer)	HRP Label (Polymer)	1 x 15′ @ 37°C
☐	Wash	ImmunoWasher	5 x 10″
☐	DAB*	DAB Chromogen*	1 x 5-10′ @ 37°C
☐	Wash	ImmunoWasher	5 x 10″
☐	Counter-stain	Hematoxylin	1 x 30″
☐	Wash	ImmunoWasher	5 x 10″
☐	Dehydrate	Alcohol	3 x 2′
☐	Prepare for Mount	Xylene	3 x 2′
☐	Mount	PermaMounter	Not Applicable

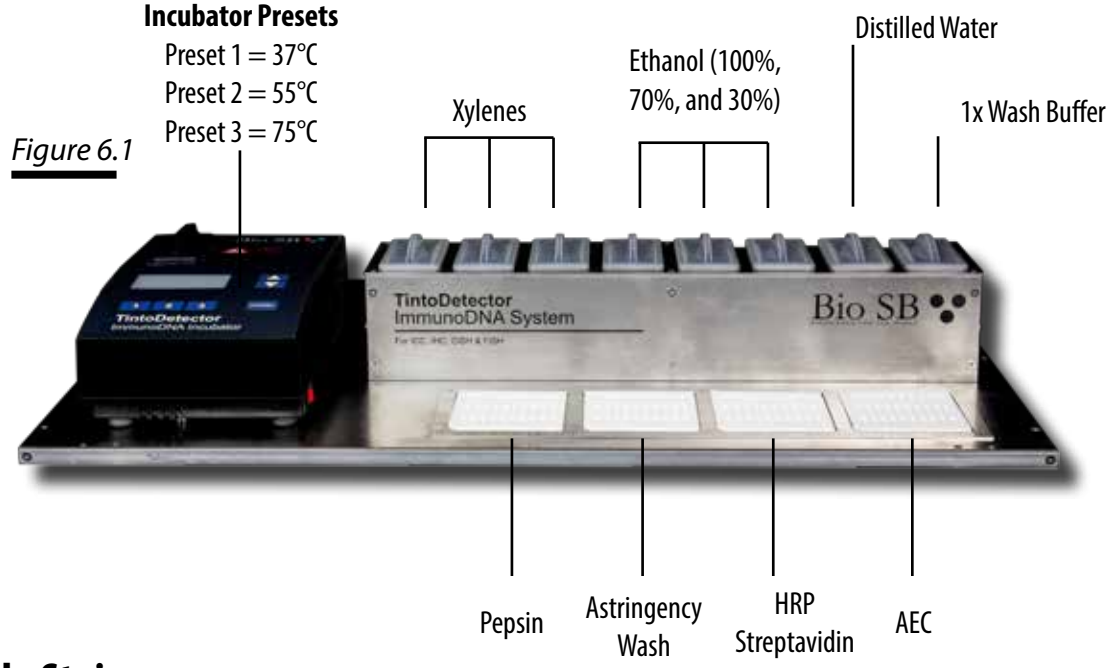
** DAB Chromogen Preparation = 1mL of Substrate : 1 mL of Chromogen. Mix and use.*

Note: Incubation times, antibody, chromogens, and kit components will vary by vendor, application, and use. Remember to read all instructions that come with detection kit to ensure accurate implementation of protocol. The protocol provided above is used solely to demonstrate the TintoDetector's ability to handle IHC.

Chromogenic In-Situ Hybridization TintoDetector Setup

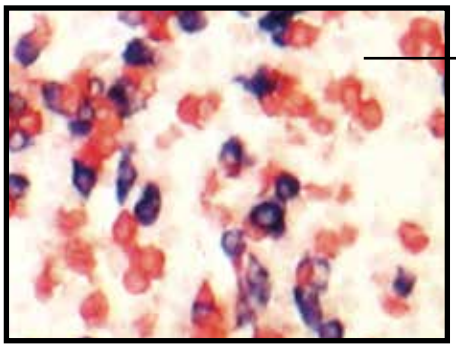
ZytoFast HRP/AEC Implementation Kit
Catalog Number: BSB 1071-40

Figure 6.1



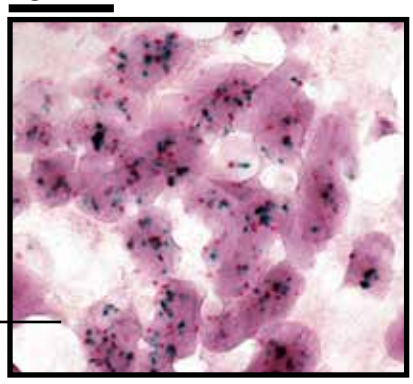
Sample Stains

Figure 6.2



Kappa Lambda
Dual Color ZytoFast
T-1017-400

Figure 6.3



HER2/CEN17
Dual Color ZytoFast
C-3032-400

Sample CISH ZytoFast HRP/AEC Sample Protocol

ZytoFast HRP/AEC Implementation Kit
Catalog Number BSB 1071-40

Note: If no temperature specified, assume room temperature.

	Step	Solution	Time/Temp (°C)
☐	1. Deparaffinize	Xylenes	2 x 3′
☐	2. Dehydrate	Alcohols	2 x 3′
☐	3. Pair Slides	Not Applicable	Not Applicable
☐	4. Digest	Pepsin Solution	1 x 25′ @ 37° C
☐	5. Wash	Distilled Water	1 x 1′
☐	6. Air Dry Slides	Not Applicable	5-10′
☐	7. Apply Probe	10 µl DNA/RNA Probe	Not Applicable
☐	8. Coverslip & Pair Slides	Not Applicable	Not Applicable
☐	9. Denature Probe	Use Incubator	1 x 5′ @ 75°C
☐	10. Hybridize	Use Incubator	1 x 60′ @ 37°C (DNA Probe) or 55° (RNA Probe)
☐	11. Soak	Wash Buffer/Coplin Jars	1 x 5′ (Until coverslips detach)
☐	12. Pair Slides	Not Applicable	Not Applicable
☐	13. Wash	1x Wash Buffer	1 x 5′ @ 55°C
☐	14. Wash	1x Wash Buffer	5 x 10″
☐	15. Detection	Mouse-Anti-Dig	1 x 30′ @ 37°C
☐	16. Wash	1x Wash Buffer	5 x 10″
☐	17. Polymer	Anti-Mouse HRP Polymer	1 x 30′ @ 37°C
☐	18. Wash	1x Wash Buffer	5 x 10″
☐	19. Chromogen	AEC Solution	1 x 15′ @ 37°C
☐	20. Wash	Distilled Water	5 x 10″
☐	21. Counter-stain	Nuclear Blue	1 x 5′
☐	22. Wash	Distilled Water	5 x 10″
☐	23. Mount	Aqueous Mounting	Mount and Air Dry

Note: Incubation times, probes, chromogens, and kit components will vary by vendor, application, and use. Remember to read all instructions that come with detection kit to ensure accurate implementation of protocol. The protocol provided above is used solely to demonstrate the TintoDetector's ability to handle CISH.

Fluorescent In-Situ Hybridization TintoDetector Setup

ZytoLight FISH Implementation Kit
Catalog Number: BSB Z-2028-20

Figure 7.1

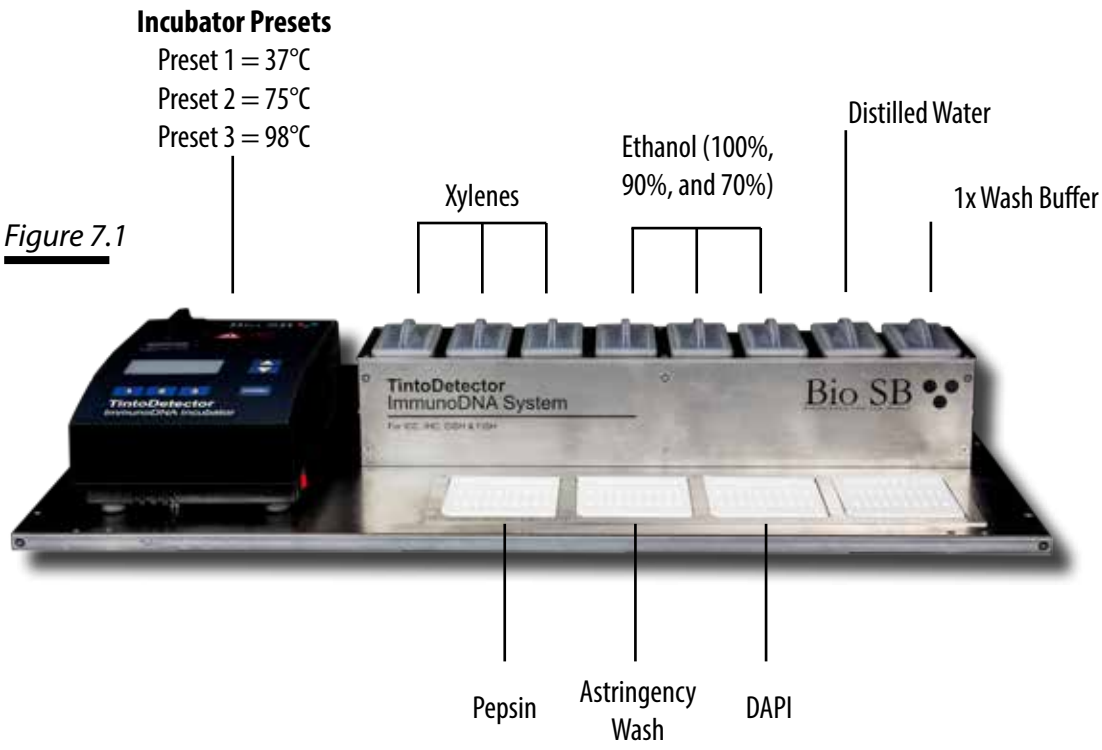
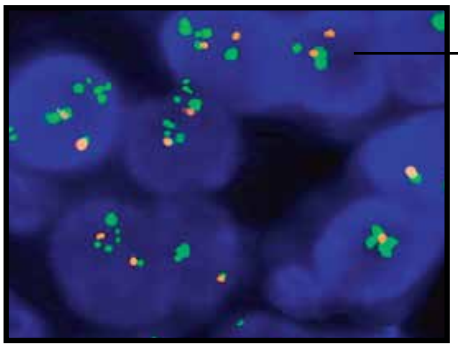
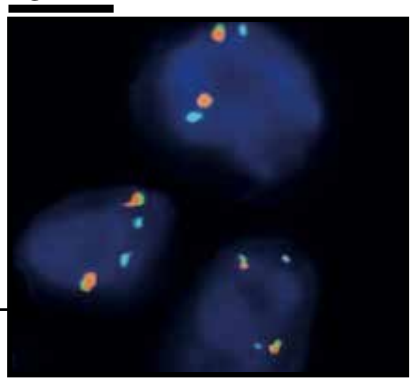


Figure 7.2



FGFR1/CEN8
Dual Color Probe
2072-200

Figure 7.3



ALK/ELM4
Tricheck Triple Color Probe
2117-200

Fluorescent In-Situ Hybridization (FISH) Sample Protocol

ZytoLight FISH Implementation Kit
Catalog Number: BSB Z-2028-20

Note: If no temperature specified, assume room temperature.

	Step	Solution	Time/Temp (°C)
☐	1. Incubate	Xylene	2 x 10′
☐	2. Incubate	100% Ethanol	2 x 5′
☐	3. Incubate	90% Ethanol	1 x 5′
☐	4. Incubate	70% Ethanol	1 x 5′
☐	5. Wash	Distilled Water	2 x 2′
☐	6. Pretreatment	Citric Solution	1 x 15′ @ 98°C in Pressure Cooker/ Water Bath
☐	7. Wash	Distilled Water	2 x 2′
☐	8. Pair Slides	Not Applicable	Not Applicable
☐	9. Apply Pepsin	Pepsin	1 x 10′ @ 37°
☐	10. Wash	1x Wash Buffer	1 x 5′
☐	11. Wash	Distilled Water	1 x1′
☐	12. Dehydrate	70% Ethanol	1 x 1′
☐	13. Dehydrate	90% Ethanol	1 x 1′
☐	14. Dehydrate	100% Ethanol	1 x 1′
☐	15. Air Dry Slides	Not Applicable	1 x 5′
☐	16. Apply Probe	10 µl FISH Probe	Not Applicable
☐	17. Coverslip & Pair Slides	Not Applicable	Not Applicable
☐	18. Denature Probe	Incubator	1 x 10′ @ 75°C
☐	19. Hybridize Probe	Not Applicable	Overnight @ 37°C
☐	20. Soak	Wash Buffer/Coplin Jar	1 x 5′ (Until coverslips detach)
☐	21. Dehydrate	70% Ethanol	1 x 1′
☐	22. Dehydrate	90% Ethanol	1 x 1′
☐	23. Dehydrate	100% Ethanol	1 x 1′
☐	24. Air Dry	Not Applicable	Not Applicable
☐	25. Apply DAPI/Antifade	30 µl DAPI/Antifade	1 x 15′
☐	26. Remove Excess DAPI	Not Applicable	Not Applicable
☐	27. Evaluate sample	Not Applicable	Not Applicable

Visit http://www.biosb.com/fish-signal-interpretation-guides/ for FISH Interpretation Guides

Note: Incubation times, probes, reagents, and kit components will vary by vendor, application, and use. Remember to read all instructions that come with detection kit to ensure accurate implementation of protocol. The protocol provided above is used solely to demonstrate the TintoDetector's ability to handle FISH.

TintoDetector Troubleshooting

Q: My TintoDetector has arrived an there are missing parts/components to the machine. How do I receive replacement parts?

A: Contact Bio SB as soon as possible, either via email, phone, or fax. Explain which part you are missing and Bio SB will gladly make arrangements to send you replacement parts.

Q: My TintoDetector has arrived and there are damaged parts.

A: Contact Bio SB as soon as possible, either via email, phone, or fax. Explain which part is damaged and Bio SB will gladly make arrangements to send you replacement parts.

Q: My solution is leaking out of the TintoDetector.

A: Inspect unit to see if the staining dishes are damaged. If a staining dish is damaged/defective, contact Bio SB for a replacement unit.

Q: Why is my solution is not drawing up the TintoDetector Slide Holder?

A: Ensure that you are using ProbeOn or ProbeOn Plus Slides with the TintoDetector Slide Holder. Also ensure that your slides are facing each other when paired, and that all slides are aligned.

Q: Bubbles form on my slides. How do I remove them?

A: We recommend that slides which form bubbles be washed in 100% Ethanol solution 5 times, followed by wash in Bio SB ImmunoWasher 10 times.

Q: My protocol isn't listed in the previous pages, can I still use the TintoDetector?

A: Yes! The TintoDetector is an open system, so you can use any IHC, ICC, CISH or FISH protocol you wish.

Q: What if I have additional questions?

A: Feel free to contact Bio SB using the following information: