INSTRUCTION MANUAL BIOSCIENCE FOR THE WORLD TINTODETECTOR IMMUNODNA SYSTEM

Compact System for Molecular Pathology





EM 0002, Rev. C DCN 4190

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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).

1. TintoDetector System (Qty: 1)	BSB 7000	
2. TintoDetector Incubator (Qty: 1)	BSB 7002	
2.1. TintoDetector Humidity Chamber (Qty.: 1)		
2.2 Power Cord (Qty.: 1)		
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	



TintoDetector Components

Incubator

See Figure 2.1

The TintoDetector Incubator is capable of reaching temperatures up to 110°C, and can store three temperature presets. The incubator is used to apply varying temperatures in CISH and FISH protocols to denature nucleic acids, probe hybridization, and to do other steps related to CISH/FISH experiments. Additionally, the TintoDetector incubator can be used to incubate any step of IHC, ICC, IF and other special stains protocols.

Power Switch

Touch Screen

See Figure 2.3

See Figure 2.2 The power switch is located at the back of the incubator.



Figure 2.2



Figure 2.3



Humidity Chamber

additional stopwatch/timer.

See Figure 2.4 The Humidity Chamber provides the necessary humidity to prevent samples from drying.

The touch screen displays all system information. There are three temperature presets. Each temperature can be programmed with a timer. The left side of the screen has an



TintoDetector Components (Continued)

TintoDetector Slide Holder

See Figure 2.5

The TintoDetector Slide Holder is a capillary gap slide holder. It fits 20 capillary gap slides. The TintoDetector Slide holder can be used for all IHC, ICC, IF, CISH, and FISH applications.





TintoDetector 30 Well Reagent Holder

See Figure 2.6

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.



Staining Dish Rack

See Figure 2.7

All TintoDetector staining dishes are capable of holding solvents and non-toxic solvents as well as any washes, buffers, or special stains for use in IHC, ICC, IF, CISH, and FISH protocols. All staining dishes will hold 200 mL of a reagent.



Installation Checklist

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





Installation Procedure

- Place a workstation on a flat- leveled surface.
- Place 30-Well Reagent Holders on a workstation.
- Place staining dishes with lids on a workstation.
- Plug incubator into an electrical outlet.
- Turn incubator on using power switch at the back of the unit.
- Make sure that display is functioning and showing the Bio SB logo (see figure 3.1)

TintoDetector Incubator Touch Screen User Manual

To Program TintoDetector incubator, preset the Incubator to the desired temperatures:

1. Fill the Humidity Chamber with 10mL distilled H₂0.

2. Click on a "temperature" **b** icon to set the desired temperature.

3. Click on the "clock" icon to set the timer. **Note:** The timer will start counting down once the incubator reaches the desired temperature. When the incubation period ends, the temperature will be lowered to 37°C. If no time is set up, the temperature will stay the same until a user click on "STANDBY" or a

"pause" 🕕 icon.

4. In order to stop incubation,press "play"		
icon. The message to confirm operation will	pop up	on
the screen as shown on <i>Figure 3.3</i> .		

5. Under the "Settings" tab a user can perform calibration and see software version and temperature unit. *See Figure3.4*



Settings

Figure 3.4

ON





A Home

Bio SB 🍤

Key Sound :

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 the 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.



TintoDetector Specifications

TintoDetector Dimensions	
TintoDetector Weight	
TintoDetector Incubator Voltage Requirements	
Supported Protocols	IHC, ICC, IF, CISH and FISH

CALIBRATION STANDARD FOR TEMPERATURE PROGRAM: ANNUALLY.

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 guidelines for the proper use of the TintoDetector System. Always reference the supplier protocol before using the TintoDetector.

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.
- Website URL: http://www.biosb.com

Support

- Email: sales@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 Slide Holder

See Figure 3.2

The TintoDetector System uses a specially designed capillary gap slide with raised white triangles at the bottom corners 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 Plus Slides is 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 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 Antibodies

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

CISH/FISH Probes and Kits

www.biosb.com/fish-cish

All CISH and FISH probes, ancillaries and reagents are compatible with the TintoDetector System. 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

Do you 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 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 Cap Gap Plus Slides with the TintoDetector Slide Holder. Also ensure that your slides are facing each other when paired, and that all slides are aligned. Make sure that lower paint triangles are not broken or damaged.

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

A: We recommend that slides 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 protocol you wish.

Q: What if I have additional questions?

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

385 Hollister Ave. Building 8, #108 Santa Barbara, CA USA 93111 Tel. (805) 692-2768 | Tel. (800) 561-1145 | Fax. (805) 692-2769 E-mail: sales@biosb.com | Website: www.biosb.com

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 Tinto-Detector offer end-users.

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

Immunohistochemistry (Biotin Detection System) TintoDetector Setup





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 or alternative	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℃
Wash	ImmunoWasher	5 x 10″
Counter-stain	Hematoxylin	1 x 30″
Wash	ImmunoWasher	5 x 10″
Dehydrate	Alcohol or ChromoProtector	3 x 2′
Prepare for Mount	Xylene or ChromoProtector	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



Sample Stains



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 or Alternative	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 or ChromoProtector	3 x 2′
Prepare for Mount	Xylene or ChromoProtector	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



Sample Stains

Figure 6.2



Kappa Lambda Dual Color ZytoFast *T-1017-400*

> HER2/CEN17 Dual Color ZytoFast C-3032-400

Figure 6.3



Sample CISH ZytoFast HRP/AEC Sample Protocol

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

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: If no temperature specified, assume room temperature.

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



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

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

Note: If no temperature specified, assume room temperature.

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.

Guarantee and Warranty

When used in laboratory conditions and according to these working instructions, this product is guaranteed for ONE YEAR from shipping date against faulty materials or workmanship. For service or technical help, please contact our Technical Support (technical@biosb.com) or your local distributor.

This product is warranted to the original purchaser only to conform to the quantity and contents stated in this manual and outer labels. Bio SB Inc.'s obligation and the purchaser's exclusive remedy under this warranty is limited to replacement, at Bio SB Inc.'s expense, of any product which shall be defective in manufacture, and which shall be returned to Bio SB Inc., transportation prepaid, or at Bio SB Inc. option, refund of the purchase price. Claims for merchandise damaged in transit must be submitted to the carrier. This warranty shall not apply to any products which have been altered outside Bio SB Inc., nor shall it apply to any products which have been subjected to misuse or mishandling.

ALL OTHER WARRANTIES EXPRESSED, IMPLIED OR STATUTORY ARE HEREBY SPECIFICALLY EXCLUDED, INCLUDING BUT NOT LIMITED TO WARRANTIES OF MERCHANT ABILITY OR FITNESS FOR A PARTICULAR PURPOSE. Bio SB Inc.'s maximum liability is limited in all events to the price of the products sold by Bio SB Inc. IN NO EVENT SHALL BIO SB INC. BE LIABLE FOR ANY SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES.

Product Name: Bio SB TintoDetector ImmunoDNA Suystem Product Catalog Number: BSB 7000

Serial No.:

This product is tested and qualified for delivery.

Checked by:

Date:



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