ImmunoBioScience Corp. (IBSC) *DATA SHEET*8070

**IBSC Mouse-on-Mouse Two-Step HRP Polymer anti-Mouse IgG (H+L) Ready-to-use IHC kit (Biotin free) with DAB chromogen and Organic Mounting medium.**

**Ready-to-use for Immunohistochemistry (IHC) and Immunocytochemistry (ICC)**

**Catalog number: IH-8070-05** Ready to use 5 ml

1. **Reagent: 5 ml= 50 tests, when 0.1 ml is applied per slide.**

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Bottle # Description

1 5 ml **Ready-to-use, Peroxidase Block, hydrogen peroxide**

(White color cap)

2 5 ml **Ready-to-use Protein Blocking solutions** (blue color cap)

3 5 ml **Mouse Endogenous blocking solution** (Pink color cap)

4 20 ml ***Primary antibody dilution buffer green color****, (for*

*Dilution of primary antibody). Please refer to the data sheet*

*of primary antibody. Use this buffer as a Negative control*

5 5 ml **Antibody Signal Enhancer (**Yellow color cap)

6 5 ml **IBSC-Two-step HRP- Polymer Secondary antibody** (Red color cap)

7 BS 5 ml **DAB buffer w/ substrate**.

7C 1 ml **DAB chromogens 20X (brown color cap)**

8 10 ml **Hematoxylin**

**9** 10 ml **Organo Mount** (Organic Mounting medium) Amber glass bottle

**-----------------------------------------------------------------------------------------------------------B. Reagents required but not supplied:** Washing buffer, antigen retrievers, positive or negative control and primary antibody.

**Description**: Immunohistochemistry (IHC)./ Immunocytochemistry (ICC) is the localization of antigens by the use of antigens in tissue sections/cells by the use of labeled antibodies as specific reagents through antigen-antibody interactions that are visualized by a marker such as fluorescent dye, enzyme, radioactive element or colloidal gold. Several IHC techniques are commonly used: labeled biotin secondary antibody streptavidin-peroxidase, HRP anti-HRP, ABC, catalyzed signal amplification, polymer system (one or two steps) and others, to detect antigens on tissue and cells. **This kit employs Polymer technology shown to provide increased sensitivity and detection.** **IBSC Two-Step Mouse-On Mouse HRP Polymer IHC kit, biotin/avidin** **free system** stains membranes, cytoplasmic and nuclear antigens. This IHC kit is designed to use anti-mouse antibodies on mouse tissues. Mouse tissue contains endogenous immunoglobulin (Ig). After primary antibody made in mouse, anti-mouse secondary antibody is applied. This antibody cannot differentiate between the endogenous Ig present on the mouse tissue and primary mouse applied in IHC. Therefore it is essential to block endogenous mouse Ig present on mouse tissue before applying primary mouse antibody. **It provides the user with a rapid and easy to use IHC** **detection system.** This kit gives best signal-to-noise ratio and since it is biotin free system tissues that are rich in Biotin do not give any background due to interaction between biotin and avidin or streptavidin.

**Intended Use**: Immunohistochemistry (IHC) and Immunocytchemistry (ICC).

**Storage**: 2-8°C, do not freeze reagents. Remove reagents from refrigerator and bring it to RT before using. After use store all reagents at 2-8°C. Positive and negative controls should be run simultaneously with the test specimens.

**Preparation of DAB Chromogen Reagent 7:** To one ml of buffer substrate (7BS) in a test tube, add two drop (50 µl) of reagent 7C (chromogen) mix well; this is ready-to-use DAB chromogen system. This reagent is good for 7-8 hours.

**Procedure: IHC/ICC procedure for frozen, paraffin sections and cell smears.**

1. Deparafinize and hydrate tissue sections through xylene or other clearing agents and graded alcohols.(For frozen sections or cell smears; use unfixed, acetone fixed or appropriate fixative for the antigen in question; **for cell smears it may be necessary to permealize the cell by detergent, please refer to antibody protocol).**
2. Wash 2-3 with distilled or deionized water.
3. Incubate sections/cell smear with **Endoblocker (#1)** for 10 minutes at room temperature (RT). Wash with distilled water 3X.
4. **Note: If antigen retriever (Trypsin AR-6541, Pronase AR-6542, Pepsin AR-6543, Citrate buffer AR-6544, Buffer w EDTA pH 8.5 AR-6545, Tris buffer pH 10 AR-6546) is required it can be applied at this step. Please refer to data sheet for the primary antibody.**
5. Wash slide with PBS or Tris saline buffer **(with 0.02-0.05% nonionic detergent, Triton X100, Tween 20 or NP-40)** or **washing buffer** (Immuno Automation buffer IBSC cat # AR-6561) 3X.
6. Incubate sections/ cell smear in **Protein blocking solution (#2),** for 5-10 minutes at RT. Do not rinse slide.
7. **Apply two drops of Mouse endogenous blocking solution (reagent #3) for 20-30 minutes at RT.**
8. Wash slide 3-5 times with washing buffer.
9. Incubate sections/cell smear with **primary antibody reagent #4 (NOT SUPPLIED\, ONLY primary** **antibody dilution buffer is supplied for dilution of Primary antibody. Incubate** for 20-30 minutes at RT. ***(For more information, refer to instructions for primary******antibody. The dilution and incubation time for Primary antibody should be determined by the investigator.****).*The primary antibody dilution buffer supplied can also be used as a negative control.
10. Wash slide with washing buff
11. Incubate slide with **Secondary antibody Enhancer (#5)** for 20 minutes at RT.
12. Wash slide 5-7 times with washing buffer.
13. Incubate slide with **IBSC-Two-step HRP- Polymer Secondary antibody reagent #6** for 15-20minutes.

**Caution: Peroxidase reagents are destroyed by sodium azide and should be avoided in all buffers and regents.**

1. Wash slide 5-7 times with washing buffer followed by distilled water.
2. Incubate with **DAB chromogen reagent #7 for 6-10** minutes at RT; monitor the color development under microscope.
3. Wash 5-7X with distilled water.
4. Incubate with **hematoxylin counterstain reagent #8** 40-60 seconds
5. Wash slide with tap water distilled water; followed by buffer with pH 7.4 -8 so that hematoxylin turns from purple to blue color.
6. Wash slide with distilled or deionized water.
7. Mount slide with **organic mounting medium (#9) (Organo Mount supplied),** by dehydrating tissue from 30% alcohol to 100% alcohol, followed by xylene or xylene substitute. Apply mounting medium, followed by coverslip, let dry at RT for few hours. **For more information , please see data sheet for Organo mount.**

These are guide lines, the optimum incubation times for these reagents and reactions should be determined by the individual lab.

**Limitation and warranty:** Our warranty is limited to the actual price paid for the product. We are not liable for any property damage, personnel injury, time, effort or economic loss due to our product.

**MSDS:** Some of these products may contain 0.05 % sodium azide as a preservative, appropriate care should be taken in handling. National Institute of Occupational Safety and Health has warning that sodium azide can react with lead, copper, brass or solder in the plumbing system and forms hydrazoic acid in acidic condition. Discard with copious amount of water. HRP reagents contain 0.05% Proclin 300. Avoid skin and eye contact with all laboratory products. Use appropriate laboratory gear, lab coats, gloves and safety glasses. Do not ingest any laboratory products. This product is not approved for administration in human or animals.

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**“*In vitro* laboratory products for research”**

Phone: + 1 425 367 4601; Fax: + 1 425 367 4817; cell (mobile) phone: + 1 425 314 0199

Marketing phone: + 1 650 343 IBSC (4272); e-mail:anitaIBSC@aol.com

Web site: [www.immunobioscience.com](http://www.immunobiosci.com); E-mail: [baderbo@gmail.com](mailto:baderbo@gmail.com)

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