ImmunoBioScience Corp. (IBSC) *DATA SHEET* **IBSC Two-Step HRP Polymer anti-Mouse, Rat and Rabbit IgG (H+L) Ready-to-use IHC kit (Biotin free) without chromogen**

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

**Catalog number: IH-8068-15** Ready to use 15 ml

**IH-8068-50** Ready to use 50 ml

1. **Reagent: 15 ml= 150 tests, and 50 ml = 500 tests, when 0.1 ml is applied per slide.**

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Bottle # (IH-8068-15) (IH -8068-50) Description

1 15 ml 50 ml Ready-to-use, Peroxidase Block, hydrogen peroxide

(White color cap)

2 15 ml 50 ml Ready-to-use Protein Blocking solutions (blue color cap)

3 25 ml 80 ml *Primary antibody dilution buffer green color, (for*

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

*of primary antibody for dilution. Use this buffer as a*

*Negative control*

*4 15 ml 50 ml Anti-Mouse, Rat and Rabbit secondary antibody Enhancer*

*(Yellow color cap)*

5 15 ml 50 ml IBSC-Two-step HRP-anti-Mouse, Rat and Rabbit IgG

(H+L) Polymer reagent. (Orange color cap)

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

**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 anti-mouse, Rat and Rabbit** IgG (H+L); biotin/avidin free system stains membranes, cytoplasmic and nuclear antigens. It is most sensitive IHC kit and provides the user with a rapid and easy to use IHC detection system.

**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

**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 5-10 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 wash slide.
7. Incubate sections/cell smear with primary antibody (NOT SUPPLIED, ONLY BUFFER IS SUPPLIED FOR DILUTION) for 20-30 minutes at RT. *(For more information, refer to instructions for primary antibody).*The primary antibody dilution buffer supplied can also be used as a negative control.
8. Wash slide with PBS 5-7X
9. Incubate with secondary antibody Enhancer (#4) for 20 minutes at RT.
10. Wash slide 5-7 times with buffer.
11. Incubate with Two-step Polymer reagent ( #5) for 30 minutes at RT.

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

1. Wash slide with deionized or distilled for 2-3X.
2. Incubate with AEC or DAB chromogen reagent (Not supplied).
3. Wash 5-7X with buffer.
4. Incubate with counterstain (Not supplied).
5. Wash slide with tap water distilled water.
6. Mount slide with appropriate mounting medium.

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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**For research use only; not for use in diagnostic procedures. FOR IN VITRO LABORATORY USE ONLY**

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

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