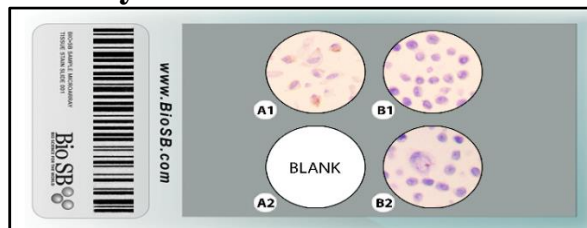


## CD117/c-kit PolyDetector HRP/DAB Detection System



**Intended Use** *For Research Use Only*

**Summary And Explanation** The **CD117/c-kit PolyDetector HRP/DAB Detection System** is a non-biotin, 2-step polymeric detection system that allows for the Immunohistochemical detection of CD117 expression in normal and neoplastic formalin-fixed paraffin-embedded tissues. The **CD117/c-kit PolyDetector HRP/DAB Detection System** has been developed using a proprietary tandem hyperlabelling technology used to directly label immunoglobulins with enzymes. This ensures consistent and reproducible immunostaining for CD117.

CD117 is a tyrosine kinase receptor for stem cell factor (SCF), also known as "steel factor" or "c-kit ligand". *C-kit* is a polypeptide that activates bone marrow precursors of a number of blood cells, but its receptor is also present on other cells. *C-kit* mutations in the interstitial cells of Cajal in the digestive tract are probably the key to gastrointestinal stromal tumors (GISTs). CD117 is found on interstitial cells of Cajal, germ cells, bone marrow stem cells, melanocytes, breast epithelium and mast cells. This receptor is found on a wide variety of tumor cells (Follicular and Papillary carcinoma of Thyroid, Adenocarcinomas from endometrium, lung, ovary, pancreas, breast; malignant melanoma, endodermal sinus tumor, small cell carcinoma) but has been particularly useful in differentiating Gastrointestinal Stromal Tumors (GIST) from Kaposi's sarcoma and tumors of smooth muscle origin.

**Presentation** The **CD117/c-kit PolyDetector HRP/DAB Detection System** contains a rabbit monoclonal CD117 prediluted antibody clone EP10, prediluted rabbit negative control, positive control slides with a normal and tumoral c-kit tissues, ImmunoRetriever with Citrate heat epitope retrieval solution, Peroxidase Blocker solution, an Anti-Rabbit Horseradish Peroxidase solution, a DAB Buffer and a DAB Chromogen solution. All the components are buffered and contain proteins, stabilizers and a non-azide anti-microbial.

### Availability

| Component Name                              | Catalog Number         |                         |                         |
|---|------------------------|-------------------------|-------------------------|
|   | BSB 0254<br>(70 Tests) | BSB 0255<br>(150 Tests) | BSB 0256<br>(500 Tests) |
| Rabbit Monoclonal CD117 Prediluted Antibody | 7 mL                   | 15 mL                   | 50 mL                   |
| Rabbit Negative Control                     | 7 mL                   | 15 mL                   | 50 mL                   |
| 3-Core GIST Cell Line Microarray            | 5 Slides               | 10 Slides               | 30 Slides               |
| 20X ImmunoRetriever with Citrate            | 50 mL                  | 100 mL                  | 200 mL                  |
| PolyDetector Peroxidase Blocker             | 15 mL                  | 30 mL                   | 100 mL                  |
| PolyDetector HRP Label                      | 15 mL                  | 30 mL                   | 100 mL                  |
| PolyDetector DAB Buffer                     | 15 mL                  | 50 mL                   | 200 mL                  |
| PolyDetector DAB Chromogen                  | 1 mL                   | 3 mL                    | 12 mL                   |

**Storage** Store at 2-8°C

**Stability** Stable up to the expiration date listed on the product label.  
Do not use this product after the expiration date listed on the product label.

### Protocol

#### Preparation of Working Solutions

The **Prediluted CD117 Rabbit Antibody**, **Prediluted Rabbit Negative Control**, **PolyDetector Peroxidase Blocker**, and **Anti-Rabbit Horseradish Peroxidase Label** are ready-to-use working solutions and require no further preparation. The **20X ImmunoRetriever with Citrate** needs to be diluted with distilled water to a 1X concentration before use by adding 50 mL of 20X ImmunoRetriever with Citrate to 950 mL of distilled water and mixing. The **DAB Chromogen** is concentrated and needs to be diluted and mixed into the **DAB Buffer** to produce the working DAB substrate-chromogen

solution. For each 1 mL of working DAB substrate-chromogen solution required for the experiment, 1 drop of **DAB Chromogen** should be added and mixed into 1 mL of **DAB Buffer**.

| Working DAB Substrate-Chromogen Required | 1 mL   | 2 mL    | 3 mL    |
|--|--------|---------|---------|
| DAB Buffer                               | 1 mL   | 2 mL    | 3 mL    |
| DAB Chromogen                            | 1 drop | 2 drops | 3 drops |

#### Recommended Immunohistochemical Protocol

1. Deparaffinize and rehydrate tissues if necessary.
2. Place cut and dried slides in the prepared **1X ImmunoRetriever Citrate** and heat treat for 15 minutes in a pressure cooker or 45 minutes in a water bath or steamer at 95°C - 98°C.
3. Wash with 5 changes of IHC Wash buffer.
4. Cover tissue with the **PolyDetector Peroxidase Blocker** for 5 min.
5. Wash with 3 changes of IHC wash buffer.
6. Cover tissue with the CD117 Primary Antibody or Negative Control and incubate for 45 minutes.
7. Wash with 3 changes of IHC wash buffer.
8. Cover tissue with the PolyDetector HRP Label, incubate for 45 min.
9. Rinse with 3 changes of IHC wash buffer.
10. Prepare DAB by adding 1 drop of **PolyDetector DAB Chromogen** per 1 mL of **PolyDetector DAB Buffer** & mix.
11. Cover tissue with the prepared DAB substrate-chromogen solution, incubate for 10 min.
12. Rinse with 5 changes of DI water.
13. Counterstain and then dehydrate.
14. Coverslip

#### Abbreviated Immunohistochemical Protocol

| Step                    | PolyDetector HRP              |
|-------------------------|-------------------------------|
| Peroxidase Blocker      | 5 minutes                     |
| Primary Antibody        | 45 minutes                    |
| HRP Label               | 45 minutes                    |
| DAB Substrate-Chromogen | 10 minutes                    |
| Counterstaining         | Time varies with counterstain |

#### Interpretation of Expected Results with 3-Core GIST Cell Line Microarray

|  |   |
|--|---|
| A1<br><b>CD117 Negative Control</b> - Normal Human Lung Fibroblast | B1<br><b>CD117 Positive Control</b> - Colorectal Adenocarcinoma |
| A2<br>BLANK  | B2<br><b>CD117 Positive Control</b> - Colorectal Adenocarcinoma |







#### Precautions

1. For professional users only. Results should be interpreted by a medical professional.
2. Always wear proper personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.
3. Minimize microbial contamination of reagents.
4. Dispose of unused solution with copious amount of water.
5. Do not ingest reagent. If reagent is ingested, contact a poison control center immediately.
6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
7. For complete recommendations for handling biological specimens please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (1).

#### References

1. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. Supplement / Vol. 61, January 6, 2012.

#### Symbol Key / Légende des symboles/Erläuterung der Symbole

|  |  |  |
|--|--|--|
|  Storage Temperature<br>Limites de température<br>Zulässiger Temperaturbereich                        |  Manufacturer<br>Fabricant<br>Hersteller              |  Catalog Number<br>Référence du catalogue<br>Bestellnummer |
|  Read Instructions for Use<br>Consulter les instructions d'utilisation<br>Gebrauchsanweisung beachten |  Expiration Date<br>Utiliser jusque<br>Verwendbar bis |  Lot Number<br>Code du lot<br>Chargenbezeichnung           |



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