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# ER/PR PolyDetector HRP/DAB Detection System

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

**Summary And Explanation**

The ER/PR PolyDetector HRP/DAB Detection System is a non-biotin, 2-step polymeric detection system that allows for the Immunohistochemical detection of Estrogen Receptor and Progesterone Receptor in tissues. The ER/PR 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 ER/PR Included in the kit are 100-core Breast Tissue Microarray (TMA) slides of formalin-fixed paraffin-embedded tissues or cells that display 0, 1+, 2+ or 3+ positivity that enables the user to quantify the ER/PR expression in tested samples.

The Estrogen and Progesterone Receptor (ER/PR) proteins are nuclear hormone receptors overexpressed in various breast carcinomas. ER and PR strongly stain the nucleus of epithelial cells. The ER and PR are important regulators of growth and differentiation of the mammary gland. The progesterone receptor (PR) is an estrogen-regulated protein. It has been proposed that expression of PR determination indicates a responsive estrogen receptor (ER) pathway.

**Presentation**

The **ER/PR PolyDetector HRP/DAB Detection System** contains rabbit ER and PR prediluted antibodies clones RBT11 and RBT22, respectively, prediluted rabbit negative control, positive control slides with Breast 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 0251 (70 Tests)** | **BSB 0252 (150 Tests)** | **BSB 0253 (500 Tests)** |
| Rabbit Monoclonal ER Prediluted Antibody | 7 mL | 15 mL | 50 mL |
| Rabbit Monoclonal PR Prediluted Antibody | 7 mL | 15 mL | 50 mL |
| Rabbit Negative Control | 7 mL | 15 mL | 50 mL |
| Positive Control Slides | 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 2-8ºC **Stability** 3 years

The PolyDetector Solutions are stable at 2-8ºC for up to **3** years from when originally produced (see expiration date on product label). These products are stable up to the expiration date on the label. Do not use this product after the expiration date. Adhere to all local laws when disposing of this product.

**Protocol** Suggested protocol on reverse

## Preparation of Working Solutions

The **Prediluted ER and PR Rabbit Antibodies, 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 95-98°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 **ER or PR 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 one drop of **PolyDetector DAB Chromogen** per 1 mL of **PolyDetector DAB Buffer** and 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 |

**Precautions** For professional users. Minimize microbial contamination of reagents. Proper handling procedures should be used.

**The following are the appropriate Risk and Safety requirements:**
Harmful if swallowed. Irritating to eyes, respiratory system, and skin. Avoid contact with skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable gloves and eye/face protection. If swallowed seek medical advice immediately and show the product container

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