

IHC Staining Procedure, Molecular Histology, Room 6-404  
 PATHOLOGY AND LABORATORY MEDICINE  
 Mount Sinai Hospital, 600 University Avenue, Toronto, On. Canada M5G 1X5

### OPTIMIZED IHC STAINING CONDITION (Validation)

Antibody Target: ER

Clone: 6F11

Cat. No: VP-E613 Lot: L1151124 Expiry Date: March 31, 2008

Source: Mouse Monoclonal (X) Rabbit Polyclonal ( ) Other:

Manufacturer: Vector Labs Supplier: Vector Labs

Isotype: IgG1; Total Volume: 1ml; Antibody Concentration: N/A Protein Concentration: N/A

Form: Concentrated Solution (X) Lyophilized Powder ( ) Prediluted Solution ( )

Storage Conditions: Unopened: Refrigerator, 2-8°C (X), Freezer, -20°C ( ), -70 °C ( )  
 Aliquots: Refrigerator, 2-8°C ( ), Freezer, -20°C (X), -70 °C ( )

Optimized Staining Conditions: Paraffin section (X), Plastic (SPURR) sections ( )

|                           | Tested Conditions | Final  |  |  |
|---------------------------|-------------------|--|--|--|
| Automated/Manual (A or M) |                   | A and M  |  |  |
| Ag Retrieval              |                   | pH 9.0 Tris-HCl Buffer<br>T/T-Mega 115 °C<br>12 min (>100 °C 6min) |  |  |
| Blocking Reagent          |                   | 1% Hs/Avidin, 10min  |  |  |
| Primary Ab                | 1:50-1:100        | 1:75 with Zymed diluent;<br>60 min                                 |  |  |
| Linking Reagent           |                   | HAM, 30 min  |  |  |
| Labeling Reagent          |                   | Elite, 30 min  |  |  |
| Chromogen                 |                   | hDAB, 10 min   |  |  |

#### Recommended Controls:

|          | Tissue        | Cell Line |
|----------|---------------|-----------|
| Positive | Breast Cancer |           |
| Negative |               |           |

Comments:

Done By: RN Sign Off by: Dr. Mullen Date: Dec 2<sup>nd</sup>, 2005

Initial Date of Use: Dec 2<sup>nd</sup>, 2005 Reviewed: April 20, 2007

Retirement Date: Reason:

Form MHL-1

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**OPTIMIZED IHC STAINING CONDITION (Validation)**

Antibody Target: PR

Clone: PgR 1294

Cat. No: M3568 Lot: 0104C Expiry Date: Oct. 2008

Source: Mouse Monoclonal (X) Rabbit Polyclonal ( ) Other:

Manufacturer: Dakocytomation Supplier: DakoCytomation

Isotype: IgG1, kappa; Total Volume: 1ml; IgG Concentration: 286ug/ml; Protein Concentration: 18.1mg/ml

Form: Concentrated Solution (X) Lyophilized Powder ( ) Prediluted Solution ( )

Storage Conditions: Unopened: Refrigerator, 2-8°C (X), Freezer, -20°C ( ), -70 °C ( )  
 Aliquots: Refrigerator, 2-8°C ( ), Freezer, -20°C ( ), -70 °C ( )

Optimized Staining Conditions: Paraffin section (X), Plastic (SPURR) sections ( )

|                              | Tested Conditions | Final  |  |  |
|------------------------------|-------------------|--|--|--|
| Automated/Manual<br>(A or M) |                   | A and M  |  |  |
| Ag Retrieval                 |                   | pH 9.0 Tris-HCl Buffer<br>T/T-Mega 115 °C<br>12 min (>100 °C 6min) |  |  |
| Blocking Reagent             |                   | 1% Hs/Avidin, 10min  |  |  |
| Primary Ab                   | 1:1000-1:2000     | 1:1000 with Zymed<br>diluent; 60 min                               |  |  |
| Linking Reagent              |                   | HAM, 30 min  |  |  |
| Labeling Reagent             |                   | Elite, 30 min  |  |  |
| Chromogen                    |                   | hDAB, 10 min   |  |  |

**Recommended Controls:**

|          | Tissue        | Cell Line |
|----------|---------------|-----------|
| Positive | Breast Cancer |           |
| Negative |               |           |

Comments:

Done By: RN Signed Off by: Dr. Mullen Date: Dec 2<sup>nd</sup>, 2005

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