**STORAGE and STABILITY**
The antibody should be stored at 2°-8°C lyophilized or reconstituted. Expiration date on vial label applies to unreconstituted product. After reconstitution, product is stable for 2 months at 2°-8°C. For extended storage, aliquot and store at -20°C or below. Avoid repeated freezing and thawing.

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PI280007 (Rev 10/08) DCC-08-1089






invitrogen™

# RB Gene Product
# Mouse Monoclonal Antibody

Catalog No. 28-0007
Formerly Ciba-Corning Diagnostics' Product No. 100601

**FOR RESEARCH USE ONLY**

Contents: 0.5 mL (Lyophi

**FORM**
The product is supplied lyophilized. Prior to filling (0.5 mL/vial) and lyophilization, the reagent contains PBS, 1% BSA, and 0.1% sodium azide. This antibody is purified and is supplied at a concentration of 100 µg/vial. This antibody has not been prepared by aseptic techniques.

**RECONSTITUTION**
Reconstitute the lyophilized material with 0.5 mL of deionized or distilled water.

**IMMUNOGEN**
Synthetic peptide corresponding to an internal region of the predicted RB protein [1].

| **CLONE** | **ISOTYPE** |
|---|---|
| **MAb 1** | Mouse $IgG_1$ |

**SPECIFICITY**
In radioimmunoprecipitation analyses, using lysates produced from the bladder carcinoma cell line T24 or the normal fetal fibroblast strain WI-38, the antibody precipitated a band of $M_r$ 110 kD with a less distinct variable region from 110kD to 116 kD. Negative reactivity was demonstrated with the retinoblastoma cell line WERI-Rb-1 and the bladder carcinoma cell line 5637.

Positive reactivity for the $M_r$ 110 kD and 110-116 kD bands were also shown by Western blot analysis of T24 and WI-38 cell lysates. Negative Western blot reactivity was demonstrated with the retinoblastoma cell line WERI-Rb-1.

Both radioimmunoprecipitation and Western blot reactivity have been blocked by antibody preadsorption with the synthetic peptide immunogen[2].

**BACKGROUND**
The retinoblastoma (RB) susceptibility gene was identified by a deletion of chromosomal region 13q14 which occurs in most childhood retinoblastomas. The loss or mutation of both alleles of the RB gene establishes that the loss of the RB gene product is the key event in the malignant transformation of retinoblasts[3]. RB gene mutations have also been demonstrated in osteosarcoma,[4-6] small cell lung carcinoma,[7,8] breast cancer,[9,10] bladder carcinoma,[11] and prostate[12] cell lines or tumors with the reintroduction of the RB gene into cultured retinoblastoma cells, the cells showed changes in growth rate, morphology, and suppression of tumorigenicity in nude mice.[13] These results suggest that the normal function of the RB gene product is to suppress proliferation.

The RB gene product has been identified by SDS-PAGE as a protein of $M_r$ 110 kD which represents the unphosphorylated RB protein. An associated, less distinct, variable region of M 110 kD to 116 kD was also identified which represents the various phosphorylated forms of the RB protein.[1] The RB protein has been shown to be modulated during the normal cell cycle.[1,14-16] Cellular localization of the RB protein has been shown by immunohistochemical staining to be primarily nuclear in the cell lines and tissues studied.[13,17,18]

The RB protein has also demonstrated the ability to form a complex with the SV 40 large (T) tumor antigen,[19] adenovirus E1A protein,[20] and the human papilomavirus type 16-E& oncoprotein.[21] These in vitro assay results suggest that the binding of these oncoproteins to the RB protein inactivates the RB protein, thus mimicking the loss of the RB gene as seen in genetic predisposition to retinoblastoma.[21]

**USAGE**
**Radioimmunoprecipitation (RIPA) of RB protein**. (Total reaction volume is 1 ML per sample) Cell cultures (1 x 10 cells/RIPA lane) are metabolically labelled with (35 S) methionine. Cells are lysed in a buffer containing 60 mM NaCl, 30mM Tris-Cl pH 7.4, 0.2% NP-40, 0.5% Na-deoxycholate, 0.1% SDS and 0.05 mg/ML aprotinin. Cell lysates are then cleared by incubations with preimmune mouse sera and Zysorbin™ (*Staphylococcus aureus cells*) or Protein A-Sepharose® with subsequent centrifugation. Cell lysates are immunoprecipitated with the RB Gene Product Monoclonal Antibody (Mab 1)

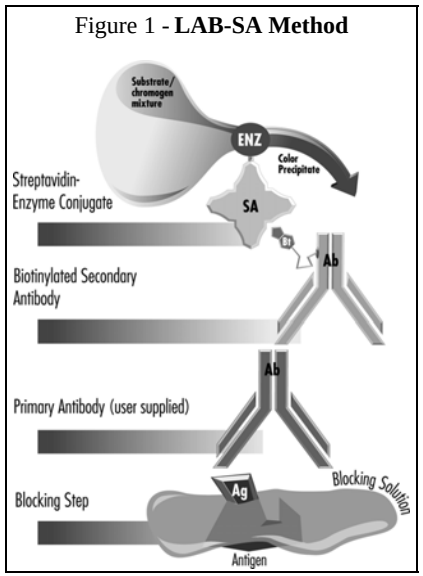
(reconstituted, undiluted antibody, 1 µg/RIPA lane) overnight at 4°C. Rabbit anti-mouse IgG is incubated with the immune complex prior to the addition of freshly prepared Protein A-Sepharose beads in PBS. Following centrifugation, the beads are washed in high salt (1M NaCl, 10mM Tris-Cl pH 7.4, 0.2% NP -40) and low salt (100 mM NaCl, 10mM Tris-Cl pH 7.4, 0.2%NP-40) washing buffers. Protein separation is accomplished by 8% SDS-PAGE.

**Western blot of RB protein**. Lyse cell cultures (2.5 x 10 cells/Wb lane) in a buffer containing 25 mM Tris-HCl pH 7.4, 50 mM NaCl, 0.5% Na-deoxycholate, 0.2% NP-40, 0.1% SDS and 0.05 mg/mL aprotinin. Following 8% SDS-PAGE, electroblot the proteins to the polyvinylidene difluoride (PVDF) membrane (0.22µ) at 10 mA for 16 hours in transfer buffer (192 mM glycine, 25 mM Tris, pH 8.3, 0.01% SDS, 20% methanol). Block with 4% BSA, 1% normal serum, 0.05% Tween-20 in TBS and then incubate with the RB Gene Product Monoclonal Antibody (Mab 1) at 0.4 µg/mL (20 mL for a 10 x 10 cm membrane overnight at room-temperature. Protein bands are detected by an alkaline phosphatase conjugated secondary antibody system.

**IMMUNOCYTOCHEMICAL STAINING**

Historically, specific demonstration of antigens have been done with fluorescent or enzymatic secondary antibody conjugates, PAP or APAAP, and ABC. Currently, the LAB-SA[22] method (also known as the Streptavidin-Peroxidase (SP) and Streptavidin-Alkaline Phosphatase (SAP) methods,[23] see Figure 1) is preferred in the immunohistochemistry laboratory due to its higher sensitivity, low background, ease-of-use, and shorter protocol times.

Figure 1 - **LAB-SA Method**




Specimens should be pre-incubated with blocking reagents to reduce nonspecific background staining. The antigen/antibody complex is then identified using the LAB-SA detection method, or other immunodetection system. The immunodetection method is supplied by the user. When using the LAB-SA method, a biotinylated secondary antibody will bind to the primary antibody that is complexed with the antigen. A streptavidin-enzyme conjugate is then added which binds to the biotinylated secondary antibody. A substrate/chromogen solution is then added that forms a colored deposit in the presence of the enzyme which is complexed to the antigen. The location of the antigen is then revealed by the presence of the colored deposit that forms around it.

The LAB-SA method is illustrated in Figure 1. The interpretation of staining or its absence should be complemented by positive and negative controls (see Controls Section ), and be performed and interpreted by qualified individuals.

| **Materials Not Provided** | **Catalog No.** | | **Catalog No.** |
|---|---|---|---|
| AEC (aminoethyl carbazole) | Invitrogen, 00-2007 | Histomount™ (mounting medium for DAB) | Invitrogen, 00-8030 |
| Antibody Diluent | Invitrogen, 00-3118 | | |
| Bovine Serum Albumin (BSA) | Sigma, A 3424 | Mayer's hematoxylin | Invitrogen, 00-8011 |
| DAB (3,3'diaminobenzidine) | Invitrogen, 00-2014 | PARA Pen | Invitrogen, 00-8855 |
| GVA (mounting medium for AEC) | Invitrogen, 00-8000 | PBS (0.01 M PBS) | Invitrogen, 00-3000 |
| HistoGrip™ | Invitrogen, 00-8050 | Sodium Azide ($NaN_3$) | Sigma, S 2002 |

Coverslips, Ethyl alcohol (EtOH, reagent grade) , Humidifying chamber, Microscope, Microscope slides, Normal serum (non-immune from secondary host), Timer, Staining jars, Xylene.

**Immunodetection Kits - Not Provided**

LAB-SA Recommended Histostain™-SP kits --available from Invitrogen:

| Chromogen | Good For | Catalog No. |
|---|---|---|
| AEC | 150 slides | 95-6543 |
| AEC | 50 slides | 95-9743 |
| AEC | 150 slides | 95-9943 |
| DAB | 50 slides | 95-9843 |
| DAB | 150 slides | 95-9643 |
| Buy Separately | 1000 slides | 95-6543-B |
| Buy Separately | 1000 slides | 95-9943-B |

PAP (Peroxidase-Anti-Peroxidase) Invitrogen, 94-8620
ABC (Avidin-Biotin Complex) Sigma, EXTRA-2

**IMMUNOHISTOCHEMICAL STAINING PROCEDURE**

**A. Tissue Section Preparation**

This procedure is for frozen tissue mounted onto a slide or cells grown on coverslips or culture slides. For paraffin-embedded tissues, we recommend using Invitrogen's mouse anti-Rb Gene Product clone Rb1 antibody (cat. no. 18-0159). Use adhesive to bond tissue to slide (i.e., HistoGrip™, Fro-Pen, or poly-L-lysine coated slides).

| | | |
|---|---|---|
| 1. | If necessary, block endogeneous biotin activity | |
| 2. | If necessary, quench endogeneous enzyme activity | |
| 3. | Block in normal serum (Reagent 1A, from Histostain™-SP kit, Ready-to-use) | 10 min. |

**B. Primary Antibody Incubation**

| | | |
|---|---|---|
| 1. | Dilute RB Gene Product antibody ~1:10 - 1:25 with diluent (10 mM PBS with 1% BSA and 0.1% $NaN3_3$, or Invitrogen Antibody Diluent Cat. No. 00-3118) | Optimal dilutions should be determined by user. |
| 2. | Incubate with diluted RB Gene Product antibody solution | Overnight incubation |
| 3. | Wash in PBS bath | 3 changes; 2 min. each |

**C. Antibody Detection,**

USING A HISTOSTAIN™-SP KIT

Positive staining of RB Gene Product in frozen tissue sections has been achieved using a Invitrogen Histostain™-SP kit with the following recommended protocol. All steps are performed at room temperature with this protocol unless otherwise indicated.

| | | |
|---|---|---|
| 1. | Incubate with biotinylated anti- mouse antibody (Reagent 1B, Ready-to-use) | 10 min. |
| 2. | Wash in PBS | 3 changes; 2 min. each |
| 3. | Incubate with streptavidin- HRP conjugate (Reagent 2, Ready-to-use) | 10 min. |
| 4. | Wash in PBS | 3 changes; 2 min. each |
| 5. | Develop with chromogen (Reagent 3A, 3B, and 3C. One drop of each into 1 ml of distilled $H_2O$) | 5 - 10 min. |
| 6. | Rinse in distilled water | 3 min. |
| 7. | Counter staining and mounting - follow kit instructions. | |

USING OTHER IMMUNODETECTION SYSTEMS

Where a commercial secondary antibody detection kit (for murine antibodies) is used, follow the manufacturer's instructions. If a commercial detection kit is not utilized, a recommended procedure is as follows:

| | | |
|---|---|---|
| 1. | Incubate with anti-mouse secondary antibody | 30 min. |
| 2. | Wash in PBS | 3 times; 2 min. each |
| 3. | Incubate with enzyme reagent (for example, ABC or PAP) | 30 min. |
| 4. | Wash in PBS | 3 times; 2 min. each |
| 5. | Develop chromogen | 5-10 min. |
| 6. | Wash in distilled water | 3 min. |

**D. Tissue Counterstaining and Preservation**

Counterstain (optional) according to manufacturer's instructions. Mount slides. Note: stability of positive staining depends on the compatibility and nature of the counterstain, mounting medium, and state of tissue (hydrated or dehydrated).

**CONTROLS**

As in any laboratory procedure the use of appropriate controls is essential. A **Positive Tissue Control** consisting of known areas of positive and negative staining for RB Gene Product should be included. A reagent control (**Negative Control-1**) should also be run in which the primary antibody step is replaced with non-specific or non-immune mouse IgG for each tissue in question. A tissue control (**Negative Control-2**) which is known not to exhibit RB Gene Product staining should also be run. The staining performance of the controls, as well as the effects of the differences in fixation, must be taken into consideration when interpreting the results by a qualified individual. Fading of stain on slides over time may occur.