

Reagents Provided

Biotinylated rhProgranulin (100 reactions): Lyophilized protein.

Avidin-Fluorescein (2 mL): Avidin conjugated with fluorescein (10 µg/mL) at an f:p ratio of 5:1.

Negative Control Reagent (60 reactions; 600 µL): A protein (soybean trypsin inhibitor) biotinylated to the same degree as the studied protein.

Blocking Antibody (15 reactions): Polyclonal goat IgG anti-human Progranulin antibody.

10X Cell Wash Buffer (2x30mL): A buffered saline-protein solution specifically designed to minimize background staining and stabilize specific binding.

Reagents Not Provided

- PBS (Dulbecco's PBS)
- human or mouse IgG

Storage

All Reagents: 2-8 °C

Intended Use

Designed to qualitatively and quantitatively determine the presence of cells expressing Progranulin receptors or other interacting proteins by flow cytometry.

Principle of the Test

Washed cells are incubated with the biotinylated protein that in turn binds to the cells via specific cell surface receptors or other interacting proteins. The cells are then directly incubated with avidin-fluorescein, which attaches to the bound biotinylated protein. Cells expressing the receptor interacting protein are fluorescently stained, with the intensity of staining proportional to the density of such protein. Relative density is then determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Biotinylated rhProgranulin: Add 1 mL of sterile distilled water to the lyophilized product. Mix gently until the product is completely dissolved. **Reagent yields optimal activity when used within 60 days of reconstitution.**

Blocking Antibody: If lyophilized, reconstitute by adding 300 µL of sterile distilled water.

1X Cell Wash Buffer: Add 1 mL of 10X concentrate to 9 mL of sterile distilled water. Reagent should be maintained on ice during use and stored at 2-8 °C for no more than 2 months.

Sample Preparation

Peripheral Blood Cells: Whole blood collected in heparinized tubes should be processed by standard Ficoll-Hypaque gradient separation techniques to isolate mononuclear cells. Ficoll and contaminating serum components should be removed by washing the cells twice with PBS and then resuspending the cells in PBS or 1X Cell Wash Buffer. Cells should be resuspended to a final concentration of 4×10^6 cells/mL.

Cultured Cells: Cells from continuous or activated cultures should be centrifuged at 500 x g for 5 minutes and then washed twice with PBS to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in PBS to a final concentration of 4×10^6 cells/mL.

Note: Adherent cell lines may require pre-treatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from the substrate should be further incubated in medium for 6-10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

Sample Staining

Add 10 µL of biotinylated protein reagent to 25 µL of the washed cell suspension in a 12 x 75 mm tube for a total reaction volume of 35 µL. As a negative staining control, an identical sample of cells should be stained with 10 µL of biotinylated negative control reagent.

1. Incubate the cells for 30-60 minutes at 2-8 °C.
2. Add 10 µL of avidin-FITC reagent to each tube. **DO NOT WASH CELLS PRIOR TO ADDING AVIDIN-FITC.**
3. Incubate the reaction mixture for an additional 30 minutes at 2-8 °C **in the dark.**
4. Wash the cells twice with 2 mL of 1X Cell Wash Buffer to remove unreacted avidin-fluorescein, and resuspend the cells in approximately 0.2 mL of 1X Cell Wash Buffer for final flow cytometric analysis.

Specificity Testing

1. (Optional) Cells that are to be stained can be pre-treated with purified mouse or human IgG (10 µL of 1 mg/mL/ 10^6 cells) for 15 minutes at room temperature in order to block Fc-mediated interactions. *Cells should not be washed of excess IgG for this assay.*
2. **In a separate tube,** 20 µL of anti-human Progranulin blocking antibody is mixed with 10 µL of Progranulin-biotin and allowed to incubate for 15 minutes at room temperature.
3. To the tube containing the anti-Progranulin blocking antibody and Fluorokine mixture, add 1×10^5 Fc-blocked cells in a volume of 25 µL.
4. The reaction is then allowed to proceed as described in steps 1-4 above.

Background Information

Progranulin, also known as Acrogranin, PC cell-derived growth factor (PCDGF) and epithelin/granulin precursor, is a ubiquitously expressed, 88 kDa, secreted glycoprotein.¹⁻³ Structurally, it does not belong to any of the well-established growth factor families. Human Progranulin is 593 amino acids (aa) in length and contains a 17 aa signal sequence and 5 potential sites for N-linked glycosylation (Swiss Prot # P28799). It has a highly repetitive organization, containing seven tandem copies of a 55-57 aa consensus motif that contains 12 conserved cysteine residues: VXCX5 6CX5CCX8CCX6CCXDX2HCCPX4CX5 6CX2.¹ There is one alternate splice form for human Progranulin. This has a deletion of aa corresponding to aa 377-531 of the standard form. Progranulin is secreted as a full length form and may undergo proteolysis leading to the release of numerous peptides made from the seven tandem repeats, called the granulins.^{2, 4, 5-7} Human Progranulin shares 75% aa sequence identity with mouse and rat Progranulin. Progranulin is involved in the regulation of cellular proliferation, as well as differentiation, development, and pathological processes.⁴ It has been isolated as a differentially expressed gene during mesothelial differentiation, macrophage development, the development of rheumatoid arthritis and osteoarthritis, sexual differentiation of the brain, and has also been shown to be a mediator of cartilage proliferation and of wound response and tissue repair.^{4, 8-13} High levels of Progranulin expression have been found to be associated with several human cancers and are believed to contribute to tumorigenesis in breast cancer, clear cell renal carcinoma, invasive ovarian carcinoma, glioblastoma, adipocyte teratoma, and multiple myeloma.^{4, 5, 12, 14-19} In addition, mutations in the Progranulin gene are a cause of frontotemporal dementia, and increased expression of Progranulin is seen in activated microglia in many neurodegenerative diseases including Creutzfeldt Jakob disease, motor neuron disease, and Alzheimer's disease.²⁰ Mutations in Progranulin causing neurodegenerative disease indicate that Progranulin is important for neuronal survival.²⁰

References

1. Plowman, G.D. *et al.* (1992) J. Biol. Chem. **267**:13073.
2. Zhou, J. *et al.* (1993) J. Biol. Chem. **268**:10863.
3. Liu, Y. *et al.* (2007) BMC Cancer **7**:22.
4. Xu, K. *et al.* (2007) J. Biol. Chem. **282**:11347.
5. Davidson, B. *et al.* (2004) Cancer **100**:2139.
6. Zanocco-Marani, T. *et al.* (1999) Cancer Res. **59**:5331.
7. Lu, R. & G. Serrero (2000) Proc. Natl. Acad. Sci. USA **97**:39.
8. Sun, X. *et al.* (2004) Am. J. Respir. Cell Mol. Biol. **30**:510.
9. Barreda, D.R. *et al.* (2004) Dev. Comp. Immunol. **28**:727.
10. Justen, H.P. *et al.* (2000) Mol. Cell Biol. Res. Commun. **3**:165.
11. Suzuki, M. & M. Nishihara (2002) Mol. Genet. Metab. **75**:31.
12. He, Z. *et al.* (2003) Nat. Med. **9**:225.
13. Zhu, J. *et al.* (2002) Cell **111**:867.
14. He, Z. & A. Bateman (2003) J. Mol. Med. **81**:600.
15. Bateman, A. *et al.* (1990) Biochem. Biophys. Res. Commun. **173**(3):1161.
16. Gonzalez, E.M. *et al.* (2003) J. Biol. Chem. **278**:38113.
17. Jones, M.B. *et al.* (2003) Gynecol. Oncol. **88**:S136.
18. Wang, W. *et al.* (2003) Clin. Cancer Res. **9**:2221.
19. Zhang, H. *et al.* (1998) Proc. Natl. Acad. Sci. USA **95**:14202.
20. Baker, M. *et al.* (2006) Nature **442**:916.

Technical Notes

This human Progranulin biotin kit has been tested for its ability to stain human TF-1 cells (see figure 1). Staining specificity has been determined by demonstrating a reduction in signal intensity when the staining reaction is carried out in the presence of a specific anti-Progranulin blocking antibody. These inhibition reactions were carried out under limiting concentrations of Progranulin-biotin. Some cell lines can exhibit high non-specific staining with labeled proteins. This effect can be compensated for by reducing the amount of labeled protein used in the reaction. We suggest that each user determine the optimal concentration of labeled protein by performing a dilution curve staining analysis on known receptor positive and negative cells. Normally, dilutions ranging from 1:2 to 1:10 are sufficient. Dilution of labeled protein should be made in 1X Cell Wash Buffer.

Typical Data

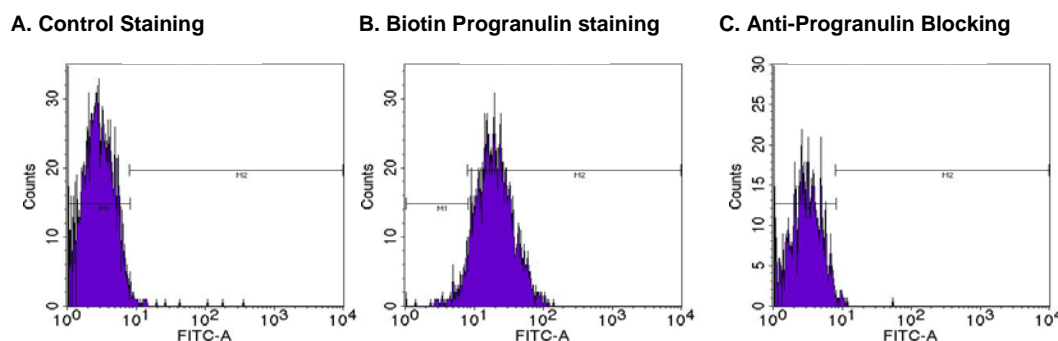


Figure 1. Human TF-1 cells were tested using this Progranulin-biotin kit. Cells stained with (A) the Negative Control protein or (B) recombinant human Progranulin-biotin are shown. Progranulin staining can be specifically blocked with (C) anti-human Progranulin antibody. Fluorescein-conjugated Avidin was used as secondary stain.

Warning: Some components in the kit contain sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal. Wear protective gloves and clothing, along with eye and face protection. Wash hands thoroughly after handling. Refer to MSDS for additional information or handling instructions.