

Reagents Provided

Biotinylated rhDkk-1 (100 reactions): Lyophilized protein.

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

600 µL Negative Control Reagent (60 reactions): A protein (soybean trypsin inhibitor) biotinylated to the same degree as the cytokine (5 µg/mL).

Blocking Antibody (15 reactions): Polyclonal goat IgG anti-human DKK-1 antibody.

60 mL 10X Cell Wash Buffer: 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 quantitatively determine the percentage of cells expressing Dkk-1 receptors within a population, and to estimate the receptor density for Dkk-1 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the biotinylated cytokine that in turn binds to the cells via specific cell surface receptors. The cells are then directly incubated with avidin-fluorescein, which attaches to the receptor-bound biotinylated cytokine. Unbound biotinylated cytokine participates in an amplification reaction with the bound cytokine that results in an enhanced signal without compromising specificity. Cells expressing the specific cytokine receptors are fluorescently stained, with the intensity of staining proportional to the density of the receptors. Relative receptor density is then determined by flow cytometry using 488 nm wavelength laser excitation.

Reagent Preparation

Biotinylated rhDkk-1: 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

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 x 10⁶ cells/mL.

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 x 10⁶ cells/mL.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from their substrates. Cells that require trypsinization to enable removal from their substrates 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 cytokine 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 could be pretreated with purified mouse or human IgG (10 µL of 1 mg/mL/10⁶ 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 DKK-1 blocking antibody is mixed with 10 µL of Dkk-1-biotin and allowed to incubate for 15 minutes at room temperature.
3. To the tube containing the anti-Dkk-1 blocking antibody and Fluorokine mixture, add 1 x 10⁵ 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

Dickkopf related protein 1 (Dkk-1) is the founding member of the Dickkopf family of secreted proteins that includes Dkk-1, -2, -3, -4, and a related protein, Soggy.^{1,2} Dkk-1 and Dkk-4 are well-documented antagonists of the canonical Wnt signaling pathway.^{1,2} This pathway is activated by Wnt engagement of a receptor complex composed of the Frizzled proteins and one of two low-density lipoprotein receptor-related proteins, LRP-5 or LRP-6.³ Dkk-1 antagonizes Wnt by forming ternary complexes of LRP-5/6 with Kremen-1 or Kremen-2.⁴ Dkk-1 is expressed throughout embryogenesis and antagonizes Wnt-7a during limb development, in developing neurons, keratinocytes, hair follicles, and the retina of the eye.⁵⁻⁹ Postnatal Dkk-1 is expressed mainly by osteoblasts and osteocytes.⁹ Both repression and expression of Dkk-1 activity may be a factor in oncogenic transformation. While activation of Wnt by repression of Dkk-1 may promote oncogenic transformation by c-myc in mammary epithelial cells or in human colon cancer,^{10,11} secretion of Dkk-1 by mesenchymal stem cells appears to inhibit cancer cell proliferation.^{12,13}

References

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Technical Notes

The human Dkk-1 biotin kit has been tested for its ability to stain the human embryonic kidney cell line HEK293 (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 1 - 100 molar excess unconjugated Dkk-1 or in the presence of the blocking antibody. These inhibition reactions were carried out under limiting concentrations of Dkk-1-biotin. Some cell lines can exhibit high non-specific staining with labeled cytokines. This effect can be compensated for by reducing the amount of labeled cytokine used in the reaction. We suggest that each user determine the optimal concentration of reduced cytokine 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 cytokine should be made in 1X Cell Wash Buffer.

Typical Data

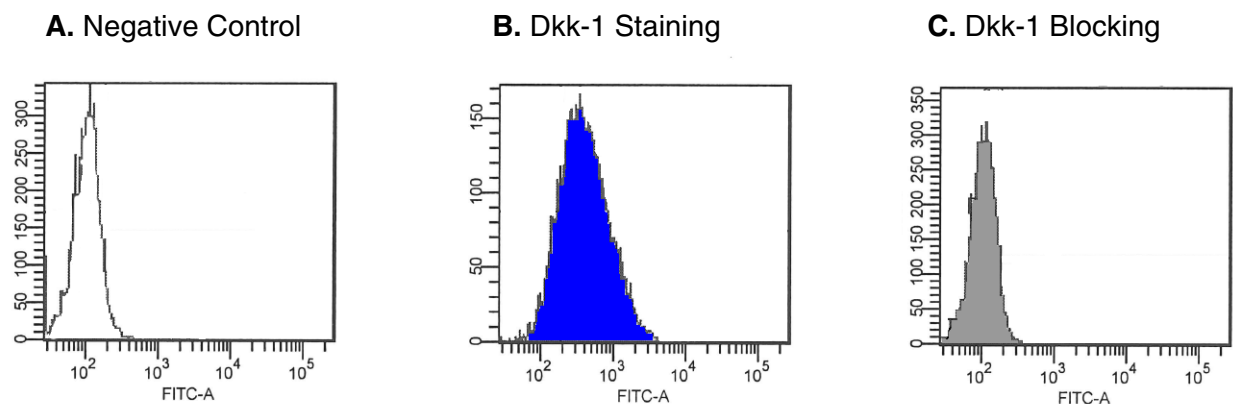


Figure 1. Human HEK293 cells were tested using the Biotinylated Human Dkk-1 Fluorokine Kit (Catalog # NFDKK1). Cells stained with the Negative Control protein (A) or with biotinylated recombinant human Dkk-1 (B) are shown. Dkk-1 staining can be specifically blocked with the anti-human Dkk-1 antibody (C). All reagents are provided with the kit.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.