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Catalog Number: GT15002

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### **Ret Staining of Spermatagonial Stem Cells Courtesy of Kim Carnes, UIUC**

1. Deparaffinize and hydration
  2. Peroxidase blocking - 30 minutes in 0.06% H<sub>2</sub>O<sub>2</sub> in methanol.
  3. Antigen retrieval - 0.01M Citrate buffer (pH6.0) in microwave (10 minutes,
    1. power level 7)
  4. Serum blocking - horse serum (unfiltered normal, diluted 1:100) for 30
    2. minutes.
  5. Primary antibody - Neuromics Ret antibody (1:200), overnight at 4° C in a
    3. moisture chamber.
  6. Secondary Ab - this was a horse universal biotinylated ab that came in a kit
    4. (with the above horse serum) diluted 1:50 with PBS and horse serum (also
    5. 1:50) for 60 minutes at room temp.
  6. ABC incubation (Vector ABC Elite kit) - 30 minutes.
  7. Colorization with DAB, counterstain with hematoxylin followed by
  8. dehydration.
- PBS used to rinse between steps.

### *FOR RESEARCH USE ONLY*

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Neuromics Antibodies • 5325 West 74<sup>th</sup> Street, Suite 8 • Edina, MN 55439  
phone 866-350-1500 • fax 612-677-3976 • e-mail: [pshuster@neuromics.com](mailto:pshuster@neuromics.com)