

**RABBIT ANTI-Nkx5.1
AFFINITY PURIFIED
POLYCLONAL ANTIBODY**

CATALOG NUMBER:	AB5744
LOT NUMBER:	21110581
QUANTITY:	100 µg
SPECIFICITY:	Recognizes Nkx5.1, (NK-2 family), a homeodomain transcription factor. The molecular weight of the protein is 49,152 Daltons.
IMMUNOGEN:	Synthetic peptide, amino acids 311-328 from Nkx5.1.
APPLICATIONS:	Western blot: 1:200-1:1,000 using ECL. Immunohistochemistry: 1:200-1:1,000. Immunocytochemistry: 1:200-1:1,000. Optimal working dilutions must be determined by the end user.
SPECIES REACTIVITIES:	Human and rodent. Reactivity with other species has not been confirmed.
FORMAT:	Affinity purified immunoglobulin.
PRESENTATION:	Precipitated antibody in a solution of 50% saturated ammonium sulfate and PBS containing no preservatives.
PREPARATION AND USE:	<p>To reconstitute the antibody, centrifuge the antibody vial at moderate speed (5,000 rpm) for 5 minutes to pellet the precipitated antibody product. Carefully remove the ammonium sulfate/PBS buffer solution and discard. It is not necessary to remove all of the ammonium sulfate/PBS solution: 10 µL of residual ammonium sulfate solution will not effect the resuspension of the antibody. Do not let the protein pellet dry, as severe loss of antibody reactivity can occur.</p> <p>Resuspend the antibody pellet in any suitable biological buffer, standard PBS or TBS (pH 7.3-7.5) are typical. Volumes required are not critical but it is suggested that the final antibody concentration be between 0.1 mg/mL and 1.0 mg/mL. For example, to achieve a 1 mg/mL concentration with 50 µg of precipitated antibody, the amount of buffer needed would be 50 µL.</p> <p>Carefully add the liquid buffer to the pellet. DO NOT VORTEX. Mix by gentle stirring with a wide pipet tip or gentle finger-tapping. Let the precipitated antibody rehydrate for 1 hour at 4-25°C prior to use. Small particles of precipitated antibody that fail to resuspend are normal. Vials are overfilled to compensate for any losses.</p>
STORAGE/HANDLING:	Maintain unopened vial at -70°C for up to 6 months. Avoid repeated freeze/thaw cycles.

The rehydrated antibody solutions can be stored undiluted at 2-8°C for 2 months without any significant loss of activity. Note, the solution is not sterile, thus care should be taken if product is stored at 2-8°C.

For storage at -20°C, the addition of an equal volume of glycerol can be used, however, it is recommended that ACS grade or higher glycerol be used, as significant loss of activity can occur if the glycerol used is not of high quality.

For freezing, it is recommended that the rehydrated antibody solution be further diluted 1:1 with a 2% BSA (fraction V, highest-grade available) solution made with the rehydration buffer. The resulting 1% BSA/antibody solution can be aliquoted and stored frozen at -70°C for up to 6 months. Avoid repeated freeze/thaw cycles.

For research use only; not for use as a diagnostic.

Important Note: *During shipment, small volumes of antibody will occasionally become entrapped in the seal of the product vial. For antibodies with volumes of 200 μ l or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

SUGGESTED PROTOCOL FOR IMMUNOHISTOCHEMISTRY

1. Dissect tissues and freeze on dry ice
2. Cut on a cryostat - 10um slices
3. Dry slides at room temperature for 30-90 min
4. Fix with cold Acetone/Methanol (50/50) 2 min - then either process further or store at -20°C .
Alternative is fixation with 4% PFA and treatment with a trypsin solution. (0.05% trypsin ,
incubation 10 minutes at 37°C, followed by 3 washes with PBS)
5. Let air dry
6. PBS 5min
7. Incubate in 50mM ammonium chloride 30 min
8. PBS 5min
9. Blocking serum 30-45min
10. Primary antibody 90min - dilution 1:100 - 1:600
11. Wash 3 times for 5 min
12. Secondary fluorescent conjug. antibody 30min
13. Wash 3 times for 5 min and mount