

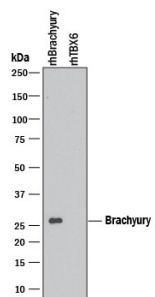
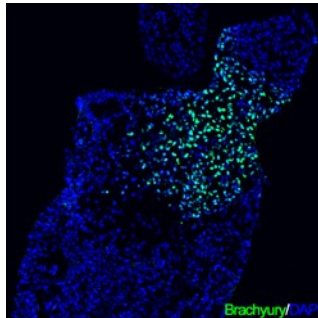
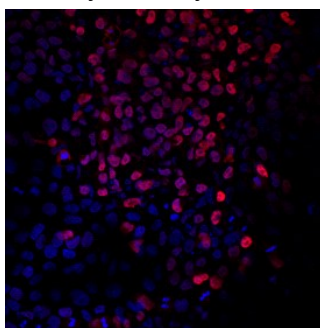
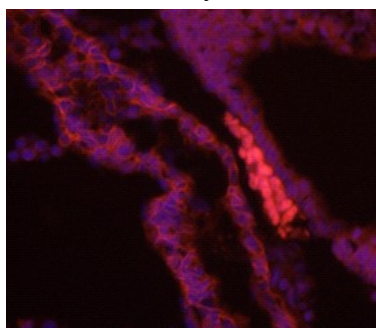
DESCRIPTION	
Species Reactivity	Human/Mouse
Specificity	Detects human Brachyury in direct ELISAs and Western blots. In direct ELISAs, less than 10% cross-reactivity with recombinant human (rh) TBX-6, rhTBX-2, rhTBX-5, and rhTBX-18 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human Brachyury Ser2-Glu202 Accession # O15178
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

APPLICATIONS

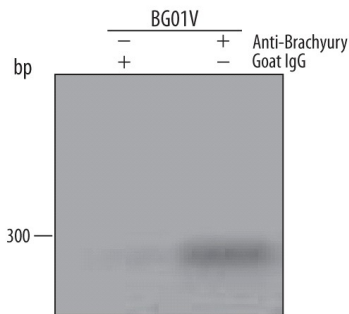
Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	0.1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Chromatin Immunoprecipitation (ChIP)	Brachyury/DNA immunocomplexes were detected in sheared chromatin from fixed BG01V Human embryonic stem cells using standard PCR for the <i>VEGF</i> promoter sequence. Use 5 µg of antibody per 5x10 ⁶ cells.	

DATA

<p>Western Blot</p>  <p>Detection of Recombinant Human Brachyury by Western Blot. Western blot shows 10 ng of Recombinant Human Brachyury and Recombinant Human TBX6. PVDF Membrane was probed with 0.1 µg/mL of Goat Anti-Human/Mouse Brachyury Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2085) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). A specific band was detected for Brachyury at approximately 26 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 3.</p>	<p>Immunocytochemistry</p>  <p>Brachyury in Differentiated Human Embryonic Stem Cells. Brachyury was detected in immersion fixed differentiated human embryonic stem cells using 10 µg/mL Goat Anti-Human Brachyury Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2085) for 3 hours at room temperature. Cells were stained (green) and counter-stained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.</p>
<p>Immunocytochemistry</p>  <p>Brachyury in BG01V Human Stem Cells. Brachyury was detected in immersion fixed BG01V human embryonic stem cells differentiated into mesoderm using Goat Anti-Human Brachyury Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2085) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the Northern-Lights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.</p>	<p>Immunohistochemistry</p>  <p>Brachyury in Embryonic Mouse Notochord. Brachyury was detected in immersion fixed frozen sections of embryonic mouse notochord (E9.5) using 10 µg/mL Goat Anti-Human Brachyury Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2085) overnight at 4 °C. Tissue was stained with the Northern-Lights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counter-stained with DAPI (blue). View our protocol for Fluorescent IHC Staining of Frozen Tissue Sections.</p>

Chromatin Immunoprecipitation (ChIP)



Detection of Brachyury-regulated Genes by Chromatin Immunoprecipitation. Mesoderm-differentiated BG01V human embryonic stem cells were fixed using formaldehyde, resuspended in lysis buffer, and sonicated to shear chromatin. Brachyury/DNA complexes were immunoprecipitated using 5 µg Goat Anti-Human/Mouse Brachyury Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2085) or control antibody (Catalog # AB-108-C) for 15 minutes in an ultrasonic bath, followed by Biotinylated Anti-Goat IgG Secondary Antibody (Catalog # BAF109). Immunocomplexes were captured using 50 µL of MagCollect Streptavidin Ferrofluid (Catalog # MAG999) and DNA was purified using chelating resin solution. The *VEGF* promoter was detected by standard PCR.

PREPARATION AND STORAGE

Reconstitution Reconstitute at 0.2 mg/mL in sterile PBS.

Shipping The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C

Stability & Storage Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 12 months from date of receipt, -20 to -70 °C as supplied.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Brachyury is the founding member of the T-box family of transcription factors, which is characterized by the N-terminal conserved DNA-binding T-domain. Brachyury is required in the early determination and differentiation of mesoderms. Human brachyury molecule shares 90% homology with mouse brachyury.

PRODUCT SPECIFIC NOTICES

BG01V cells are licensed from ViaCyte, Inc.