**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**  
E. coli-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**  

<table>
<thead>
<tr>
<th>Sample</th>
<th>Western Blot</th>
<th>Immunocytochemistry</th>
<th>Immunohistochemistry</th>
<th>Chromatin Immunoprecipitation (ChIP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 µg/mL</td>
<td>5-15 µg/mL</td>
<td>5-15 µg/mL</td>
<td>Brachyury/DNA immunocomplexes were detected in sheared chromatin from fixed BG01V Human embryonic stem cells using standard PCR for the VEGF promoter sequence. Use 5 µg of antibody per 5x10⁶ cells.</td>
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</table>

**DATA**

**Western Blot**

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.

**Immunocytochemistry**

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 counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

**Immunohistochemistry**

Brachyury in Embryonic Mouse Notochord. Brachyury was detected in immersion fixed 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 using the Northern-Lights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). View our protocol for Fluorescent IHC Staining of Frozen Tissue Sections.
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 MagCellect 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.