

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 as a 0.2 µm filtered solution in PBS.

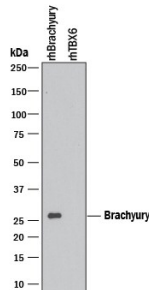
APPLICATIONS

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.	

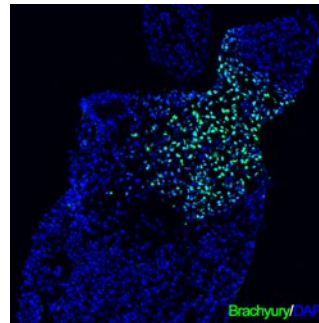
DATA

Western Blot



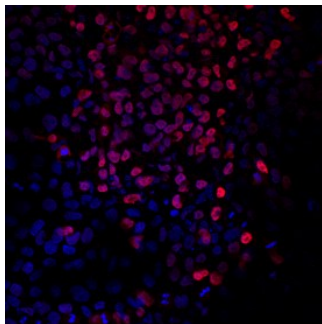
Detection of Recombinant Human Brachyury by Western Blot. Western blot shows 10 ng of Recombinant Human Brachyury and Recombinant Human TBX6. PVDF Membrane was probed with 0.1 µg/mL of Goat Anti-Human/Mouse Brachyury Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2085) followed by HRP-conjugated 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



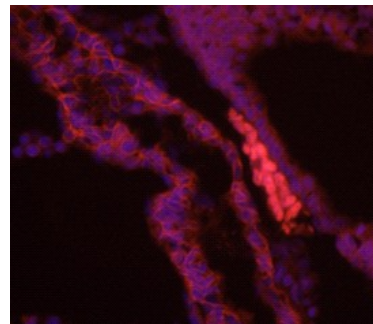
Brachyury in Differentiated Human Embryonic Stem Cells. Brachyury was detected in immersion fixed differentiated human embryonic stem cells using 10 µg/mL Goat Anti-Human Brachyury Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2085) for 3 hours at room temperature. Cells were stained (green) and counterstained with DAPI (blue). View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunocytochemistry



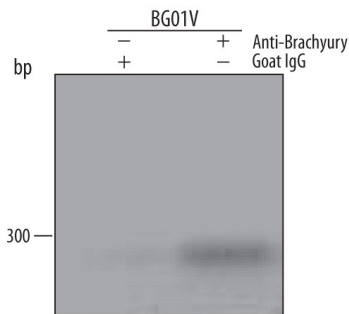
Brachyury in BG01V Human Stem Cells. Brachyury was detected in immersion fixed BG01V human embryonic stem cells differentiated into mesoderm using Goat Anti-Human Brachyury Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2085) at 10 µg/mL for 3 hours at room temperature. Cells were stained 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](#).

Immunohistochemistry



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 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 MagCelect 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. <ul style="list-style-type: none"> ● 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.