

# Immunohistochemistry Using Anti-TGF-Beta 1 Polyclonal Antibody

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*Promega's Anti-TGF-Beta 1 Polyclonal Antibody\* successfully detected TGF-Beta 1 in formalin-fixed neonatal rat kidney samples. Antigen detection was dependent on pronase E treatment and overnight incubations with primary antibody. Formalin-fixed tissues produced stronger signals when compared to unfixed samples.*

*\* TGF-Beta 1 products are for research use only and are not for use in diagnostic procedures.*

## Introduction

The use of polyclonal and monoclonal antibodies to detect both the presence and location of antigens in tissue has become commonplace in today's laboratories. While interest in new cytokines, growth factors, oncoproteins and other peptides has diversified considerably, the repertoire of available antibodies capable of detecting these antigens in tissue has often limited their investigation.

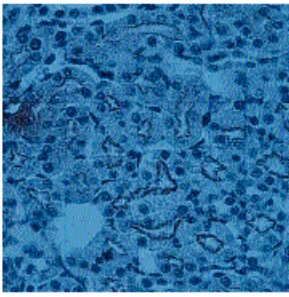
Recently, the transforming growth factor beta 1 (TGF-Beta 1) has been investigated in numerous model systems (for reviews, see 1-2). TGF-Beta 1 has been associated with intracellular matrix deposition and tissue repair/damage, cell cycle control and programmed cell death or apoptosis. TGF-Beta 1 is a protein of immense interest to a number of fields, but scientific studies of the protein have been either indirect or at the mRNA level. Until recently, the availability of a useful antibody with sufficient specificity has precluded immunochemical studies of TGF-Beta 1. Also, the widespread use of formalin fixatives makes many clinical samples inappropriate for the use of previously available TGF-Beta 1 antisera. Promega's Anti-TGF-Beta 1 Polyclonal Antibody is capable of detecting TGF-Beta 1 in rat kidneys using a variety of fixatives, most notably formalin.

## Immunohistochemistry procedure for TGF-Beta 1 detection

Sprague Dawley rat kidneys from adults or neonates were harvested and either dehydrated through graded alcohols and xylene or fixed in 4% neutral buffered formalin for 4 hours at 4°C prior to embedding in paraffin. Seven micron sections were cut and adhered to silanized slides (Sigma). Sections were hydrated through xylene and graded alcohols into phosphate buffered saline (PBS) and treated for 8 minutes with either 0.1% pronase E (Sigma) or Biomeda's pronase solution. Sections were rinsed twice in distilled H<sub>2</sub>O for 5 minutes and quenched in 0.6% H<sub>2</sub>O<sub>2</sub>-methanol. Slides were rinsed again, blocked for 20 minutes with room temperature goat serum (Vector Laboratories) and incubated with primary antibody overnight at 4°C. The primary antibody was either a 1:50 dilution of Promega's rabbit Anti-Human TGF-Beta 1 pAb (prepared fresh) or a 1:50 dilution of Promega's Anti-Human TGF-Beta 1 pAb, which had been preadsorbed with 0.5µg of Promega's TGF-Beta 1 protein (Cat.# G1241) for 1 hour at room temperature. The samples were then rinsed in PBS, incubated with dilute biotinylated anti-rabbit IgG (Vector Laboratories) for 30 minutes, rinsed again, and finally incubated with avidin-biotin complex (Vector Laboratories) for 1 hour at room temperature. DAB (Sigma) was used as the chromagen for 7 minutes. Gill's #2 Hematoxylin (Sigma) was used as the counterstain. Sections were dehydrated through graded alcohols and xylene and mounted with Permount.

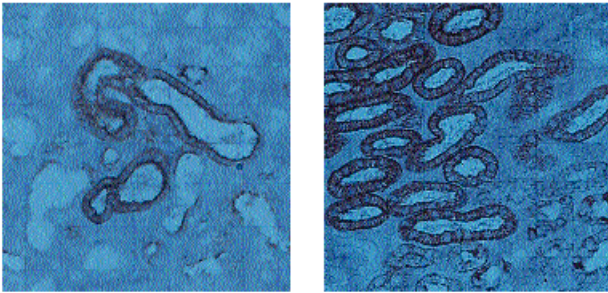
## TGF-Beta 1 localization in neonate and adult kidneys

TGF-Beta 1 staining was visualized in two general locations of the rat kidney. Formalin-fixed and unfixed neonate kidney samples showed positive staining in the lumen side of tubules and in vessel walls. Tubule staining is distinguished as a thin dense band around the inside border ([Figure 1](#)). Formalin-fixed and unfixed adult kidney specimens displayed positive staining in tubules as well, however, it was present on both the apical side and the basolateral side of the tubule ([Figure 2](#)). In both adults and neonates, staining was seen exclusively on the cell surface with faint detection in the cytosol. While positive staining was detected with both formalin-treated and unfixed tissue, the use of a fixative yields better results. Unfixed tissue has a typically distorted morphology compared to fixed sections. Additionally, unfixed sections tend to dissolve during pronase treatment. Serial formalin fixed sections treated with pre-adsorbed antisera displayed no appreciable staining ([Figure 3](#)).

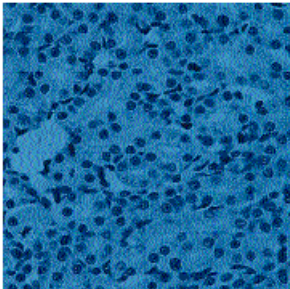


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**Figure 1. Formalin-fixed 14-day neonatal rat kidney tissue section treated with Anti-TGF-Beta 1 Polyclonal Antibody.** Methods are described in the text. The image was captured at 200x magnification.



**Figure 2. Unfixed adult rat kidney tissue section treated with Anti-TGF-Beta 1 Polyclonal Antibody.** Methods are described in the text. The image was captured at 200x magnification.



**Figure 3. Formalin-fixed 14 day neonatal rat kidney tissue section treated with serial, pre-adsorbed Anti-TGF-Beta 1 Polyclonal Antibody.** Methods are described in the text. The image was captured at 200x magnification. Note the absence of Anti-TGB-Beta 1 binding to the lumen side of the tubules.

## Critical parameters for immunohistochemical detection of TGF-Beta 1

Numerous conditions were tested to detect the presence of TGF-Beta 1 in rat kidneys using the Anti-TGF-Beta 1 pAb. From the results, we concluded that positive staining is primarily dependent upon pronase E digestion and overnight primary antibody incubations.

A number of other conditions were attempted, but none demonstrated any increase in TGF-Beta 1 detection sensitivity over the procedure described. These conditions include: 1) substituting the pronase treatment with either a 2-15-minute Proteinase K (20µg/ml) treatment at room temperature or a 5-minute trypsin treatment; 2) heating in a microwave for up to 10 minutes in 10mM sodium citrate, pH 6; 3) primary antibody incubation for 1-3 hours at room temperature or 37°C; 4) longer primary incubations with more dilute antibody (e.g., 24-36 hours with 1:75-1:100 dilutions; 5) using prediluted primary antibody aliquots (i.e., antibody dilution must be prepared fresh each time); and 6) 2-hour fixation in Bouin's reagent at room temperature.

## Summary

Promega's Anti-Human TGF-Beta 1 Polyclonal Antibody was used to localize TGF-Beta 1 in rat kidneys. Successful TGF-Beta 1 detection in these tissues depended primarily on pronase E treatment and overnight primary antibody incubations. The data demonstrate that Promega's Anti-TGF-Beta 1 Polyclonal Antibody also detects TGF-Beta 1 in formalin-fixed kidney sections, overcoming a problem with previously available TGF-Beta 1 antisera.

## References

1. Massague, J. (1990) *Ann. Rev. Cell. Biol.* **6**, 597.
2. Okragly, A., Balwit, J.M., and Haak-Frendscho, M. (1994) *Promega Notes* **47**, 10.

## Ordering Information

Product	Size	Cat. #
Anti-TGF-Beta 1 pAb	100µg	G1221
TGF-Beta 1, Human, Natural	2µg	G1241