Datasheet for 200-201-A26

SIX3 Antibody

Overview

Description:	Anti-Homeobox protein SIX3 (GUINEA PIG) Antibody - 200-201-A26
Item No.:	200-201-A26
Size:	50 µg
Applications:	Dot Blot, IF, IHC, WB, Multiplex
Reactivity:	Mouse
Host Species:	Guinea Pig

Product Details

Background:	Six3 (also known as sine oculis homeobox homolog 3) is involved in the development of the visual system and forebrain. Six3 is a nuclear protein that is reported to exist in two forms by alternative splicing of the gene product. Six3 is first expressed at E6.5 of mouse embryonic development around the anterior border. At E8.5, expression is found over the anterior neural plate. At E9.5, it is in the diencephalic part of the ventral forebrain, optic vesicles, olfactory placodes and Rathke's pouch. In later stages, Six3 is present in hypothalamus, eyes and pituitary.
Synonyms:	guinea pig anti-SIX 3 antibody, Six3, Sine oculis homeobox homolog 3
Host Species:	Guinea Pig
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	Six3
Reactivity:	Mouse
Immunogen Type:	Conjugated Peptide
Immunogen:	This Protein A purified antibody was prepared from whole guinea pig serum produced by repeated immunizations with a synthetic peptide corresponding to an internal region of mouse Six3 protein.

Purity/Specificity:	This product was purified by Protein A chromatography from monospecific antiserum. This antibody reacts with mouse Six3. Cross-reactivity with Six3 from other sources has not been determined.
Relevant Links:	• UniProtKB - Q62233 • NCBI - 59939908 • GeneID - 20473

Application Details

Tested Applications:	Dot Blot, IF, IHC, WB
Suggested Applications:	Multiplex (Based on references)
Application Note:	This Protein A purified antibody has been tested for use in Immunohistochemistry, immunofluorescence microscopy, dot blot, and western blotting. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 37 kDa in size corresponding to Six3 by western blotting in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:5,000 - 1:25,000
IF:	1:250 - 1:500
IHC:	1:250 - 1:500
WB:	1:500 - 1:2,000

Formulation

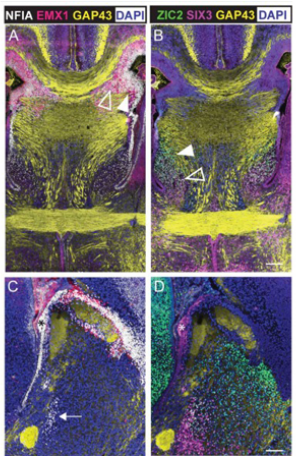
Physical State:	Liquid (sterile filtered)
Concentration:	0.65 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

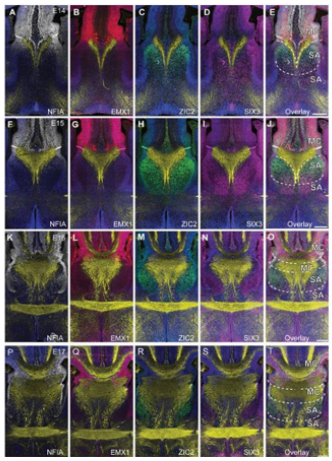
Shipping Condition:	Dry Ice

Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

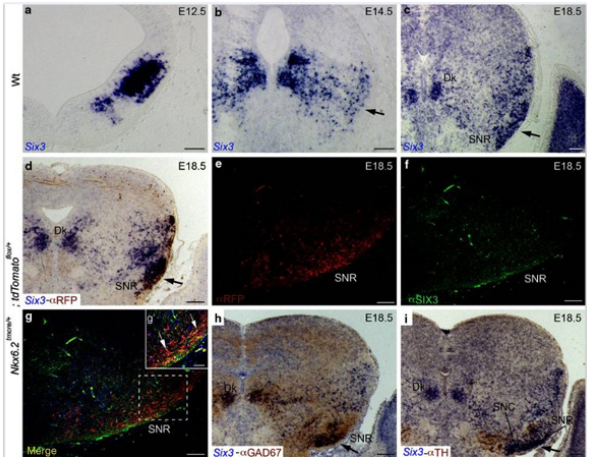
Images



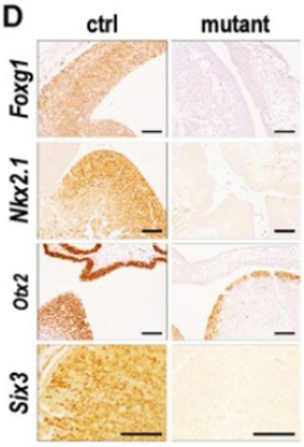
Immunofluorescence Microscopy
Molecular delineation of the mouse commissural plate at E17 reveals four subdomains. Fluorescence immunohistochemistry of the dorsal telencephalic markers NFIA (closed arrowhead, A) and EMX1 (open arrowhead, A) at E17 in the oblique coronal plane and sagittal plane, where two (5µm think) adjacent sections have been overlaid (A, C). The ventral telencephalic markers SIX3 (open arrowhead) and ZIC2 (closed arrowhead) are expressed in the same sections (B, D). GAP43 labeling is shown in yellow to delineate the commissural axons. DAPI counterstain is shown in blue. While both NFIA and EMX1 encircle the rostral to caudal extent of the CC and surround the DHC, only NFIA expression spans the commissural plate ventrally to end rostral and dorsal of the AC (arrow). From the VHC towards the AC, the ventral markers ZIC2 and SIX3 are expressed in opposing gradients. ZIC2 is predominantly expressed around the fornix, while SIX3 is predominantly expressed around the AC. Laterally, ZIC2 is expressed within the septum, bounded by the lateral ventricles. However, SIX3 is expressed along the mediolateral extent of the AC. Immunoreactivity is also present in the choroid plexus and the epithelium of the rostral wall of the third ventricle (asterisks in C and D). These features are not considered part of the commissural plate. From these expression patterns dorsal domains (MC) and ventral domains (SA) are characterized as follows: MC or MC1, NFIA+ and EMX1+; MC2 NFIA+ and ZIC2+; SA1, NFIA+ and ZIC2+ and SIX3+; SA2, SIX3+. Scale bars: A-B = 200 µm, C-D = 150 µm. Fig 3.
PMID: 20653027



Immunofluorescence Microscopy
Molecular patterning and morphology of the developing mouse commissural plate demonstrates the emergence of the four subdomains. Fluorescence immunohistochemistry for the markers NFIA (white), EMX1 (red), SIX3 (magenta) and ZIC2 (green) throughout the developmental oblique coronal plane of the commissural plate in mouse embryos at E14 (A-E), E15 (F-J), E16 (K-O) and E17 (P-T). GAP43 staining is shown in yellow. DAPI counterstain is shown in blue. An overlay of this molecular patterning is shown with boundaries indicated by dashed lines, defining dorsal domains (MC) and ventral domains (SA), characterized as follows: MC or MC1, NFIA+ and EMX1+; MC2 NFIA+ and ZIC2+; SA1, NFIA+ and ZIC2+ and SIX3+; SA2, SIX3+. Scale bar = 200 µm. Fig 4.
PMID: 20653027

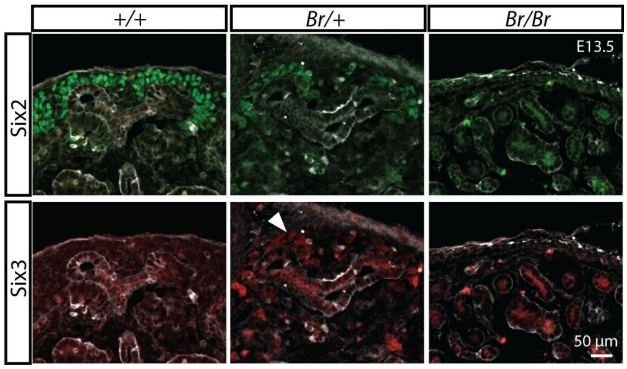


Immunofluorescence Microscopy
Midbrain Six3 expression pattern. Selected transversal paraffin sections through embryos at different stages. Six3 in situ hybridization at E12.5 (a), in E14.5 (b) and in E18.5 (c). The arrows in b and c indicate the area of SNR. d E18.5 Six3 in situ hybridization in blue combined with α-RFP in brown, the arrow indicates the SNR. e, f E18.5 immunofluorescent reacted against α-RFP in red (Nkx6.2 derivatives) and α-SIX3 in green. g Combined e and f, showing the coexpression between Nkx6.2 derivatives and positives Six3 neurons. g' A high magnification of g to better illustrate the colabelling, indicated by arrows. Cells expressing Six3 and Nkx6.2 contribute to the SNR. h, i α-GAD67 and α-TH in brown, respectively. The arrows in h, i indicate the SNR. Dk Darkschewitsch nucleus, SNC Substantia nigra pars compacta, SNR Substantia nigra pars reticulata. Scale bars 100 µm in a, b, g'; 200 µm in c–i.
Fig 5. PMID: 25579066

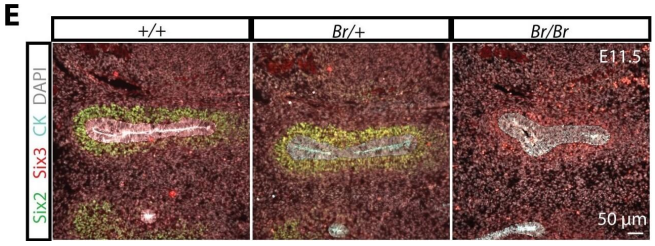


Immunohistochemistry
C) Transcription factor immunodetection in parasagittal head sections of E13.5 control and mutant embryos. Bars: 100 µm. Fig 4. PMID: 31649239

D



Immunohistochemistry
The Six2 regulatory landscape is altered in the Br mouse leading to reduced Six2 expression and ectopic Six3 expression in the kidney.(A) Schematic showing the X-irradiation induced breakpoints and subsequent deletion with inversion that resulted in the Br allele. LE = Lens enhancer (putative), PE = proximal enhancer, DE = distal enhancer. Black box between the Six2 and Six3 loci represents the predicted boundary. (B) Interaction matrix (top) generated by Hi-C data (Hardison lab hESC Hi-C data, http://promoter.bx.psu.edu/hi-c/view.php). Genomic view showing Six2 ChIP-seq, CTCF-NP ChIP-seq, and 4C-seq data (bottom). The region inverted in the Br allele is highlighted. Dashed square indicates a predicted TAD boundary element that lies between Six3 and Six2 loci [70]. (C) qPCR showing the relative expression levels of Six2 and Six3 in E13.5 kidneys of the indicated genotype. (D) E13.5 kidneys of the indicated genotype were sectioned and immunostained for Six2 and Six3. Arrow points to the low level Six3 expression in nephron progenitors. (E) Immunostaining for Six2, Six3, cytokeratin (CK), and DAPI in E11.5 kidneys of the indicated genotype. Figure provided by CiteAb. Source: PLoS Genet, PMID: 29377931.



Immunohistochemistry

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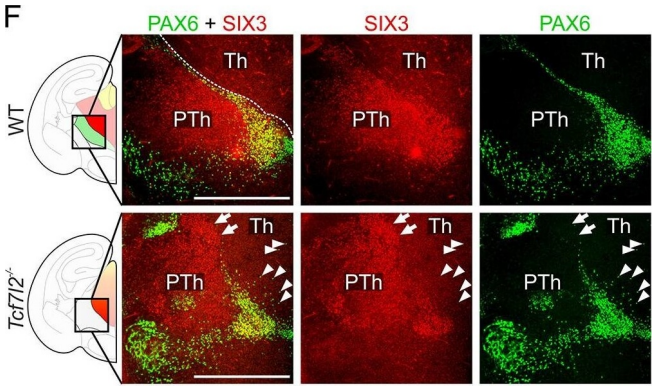
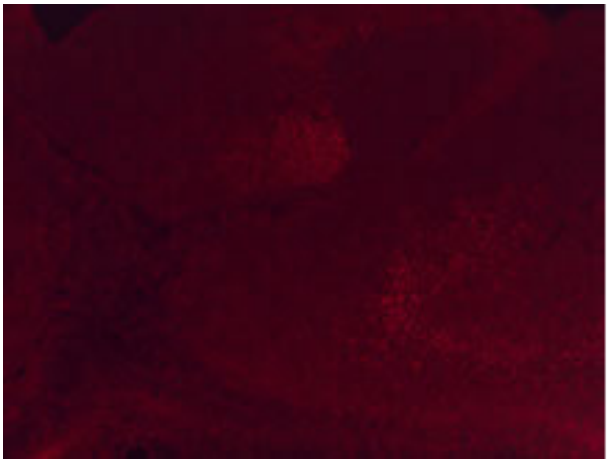


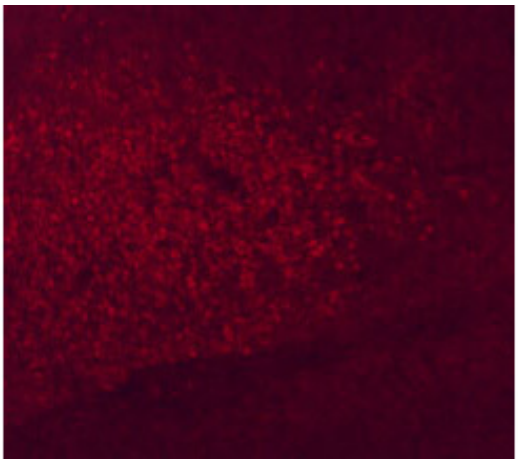
Immunohistochemistry

TCF7L2 controls the establishment of anatomical borders in the thalamus and habenula. (A) In situ hybridisation with a Tcf7l2 probe in consecutive E18.5 coronal brain sections. (B-D) In situ hybridisation with Gbx2 (B), Nkx2-2 and Sox14 (C) and Pax6 (D) probes in E18.5 coronal brain sections. Schematic above indicates areas shown in micrographs below. (E) Immunofluorescent co-staining of PAX6 and TCF7L2 (WT embryos) or β-galactosidase (Tcf7l2−/− embryos) in E18.5 coronal brain sections. White arrowheads show PAX6-positive cells invading the thalamus in Tcf7l2−/− embryos. (F) Immunofluorescent staining of PAX6 and SIX3 in E18.5 coronal brain sections. White arrows show SIX3-positive cells and white arrowheads show PAX6-positive cells which intermingle into the thalamic region. (G) Immunofluorescent co-staining of NKX2-2 and TCF7L2 (WT mice) or β-galactosidase (Tcf7l2−/− mice) in E18.5 coronal brain sections. White arrowheads show NKX2-2-positive cells from the rostral thalamus invading the thalamus in Tcf7l2−/− embryos. (H) Immunofluorescent staining of POU4F1 in coronal brain sections. White arrowheads show POU4F1-positive cells spreading into the thalamic area. Dotted lines demarcate different regions. Magnification of boxed areas are shown as indicated. Cx, cortex; Hb, habenula; lHb, lateral habenula; mHb, medial habenula; Hp, hippocampus; Pt, pretectum; PTh, prethalamus; rTh, rostral thalamus; Th, thalamus; Th-Hb, thalamo-habenular region. Scale bars: 0.5 mm. Figure provided by CiteAb. Source: Development, PMID: 32675279.

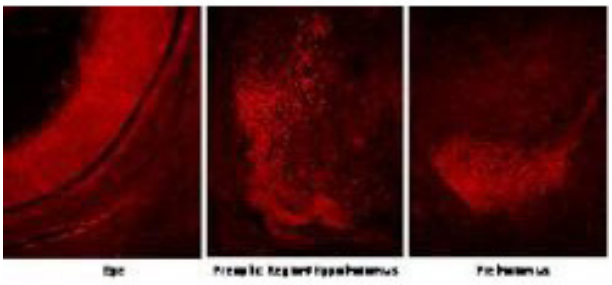


Immunofluorescence Microscopy

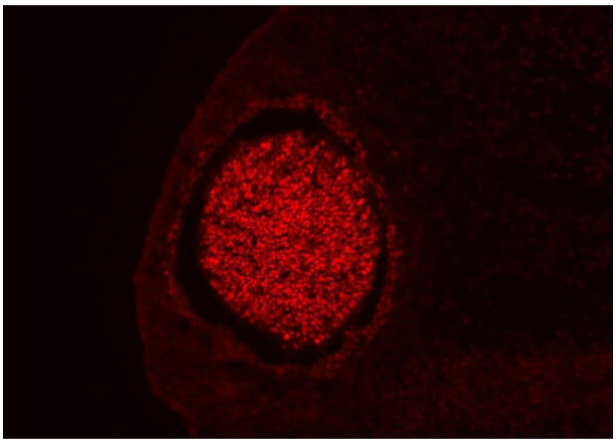
Immunofluorescence microscopy using Rockland's anti-Six3 antibody shows detection of Six3 in PFA-fixed embryonic mouse hypothalamus. Primary antibody was used at 1:250 dilution. Personal Communication, Miriam Dillard, St. Jude Children's Research Hospital, Memphis, TN.



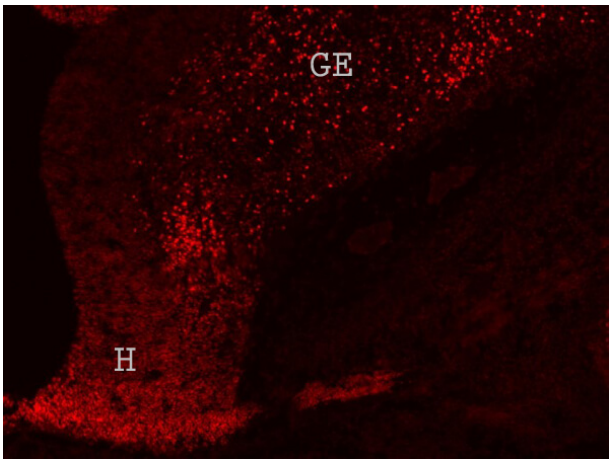
Immunofluorescence Microscopy
Immunofluorescence microscopy using Rockland's Anti-Six3 antibody shows detection of Six3 in PFA-fixed embryonic mouse prethalamus. Primary antibody was used at 1:250 dilution. Personal Communication, Miriam Dillard, St. Jude Children's Research Hospital, Memphis, TN.



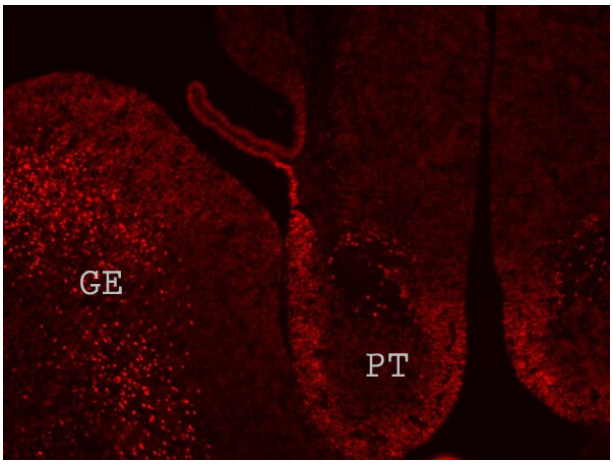
Immunofluorescence Microscopy
Immunofluorescence microscopy using Rockland's Protein A purified anti-Six3 antibody shows detection of Six3 in various E14.5 mouse embryonic tissues. Tissue was fixed using 4% PFA. The primary antibody was used at a 1:200 dilution. Personal Communication, A. Lavado, St Jude Children's Research Hospital, Memphis, TN.



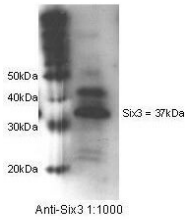
Immunofluorescence Microscopy
Immunofluorescence of Guinea Pig Anti-Six 3 Antibody. Tissue: E12.5 mouse embryonic eye. Fixation: PFA fixed and embedded in OCT/Tissue Tek for cryo. Antigen retrieval: not required. Primary antibody: SIX3 antibody at 1:500 for overnight at RT. Secondary antibody: Cy3 secondary antibody at 1:250 for 3hrs. Localization: nuclear. Staining: antibody visualized as red signal.



Immunofluorescence Microscopy
Immunofluorescence of Guinea Pig Anti-Six 3 Antibody. Tissue: E12.5 mouse embryonic eye: ganglionic eminence (GE) and hypothalamus (H). Fixation: PFA fixed and embedded in OCT/Tissue Tek for cryo. Antigen retrieval: not required. Primary antibody: SIX3 antibody at 1:500 for overnight at RT. Secondary antibody: Cy3 secondary antibody at 1:250 for 3hrs. Localization: nuclear. Staining: antibody visualized as red signal.



Immunofluorescence Microscopy
Immunofluorescence of Guinea Pig Anti-Six 3 Antibody. Tissue: E12.5 mouse embryonic eye: ganglionic eminence (GE) and prethalamus (PT). Fixation: PFA fixed and embedded in OCT/Tissue Tek for cryo. Antigen retrieval: not required. Primary antibody: SIX3 antibody at 1:500 for overnight at RT. Secondary antibody: Cy3 secondary antibody at 1:250 for 3hrs. Localization: nuclear. Staining: antibody visualized as red signal.



Western Blot
Western blot using Rockland's Protein A purified anti-Six3 antibody shows detection of Six3 in whole cell lysate. The band marked corresponds to Six3 at an approximate molecular weight of 37Kda. The primary antibody was used at a 1:1000 dilution. Personal Communication, N. Johnson, St Jude Children's Research Hospital, Memphis, TN.

References

- Lipiec MA et al. TCF7L2 regulates postmitotic differentiation programmes and excitability patterns in the thalamus. *Development.* (2020)
- Grall, E et al. Severe head dysgenesis resulting from imbalance between anterior and posterior ontogenetic programs. *Cell Death & Disease* (2019)
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