CORTICAL LAYERS

The cerebral cortex is the part of the human brain that undergoes the most profound evolutional changes and serves as a substrate for higher cognitive functions.

During embryonal development, six distinct layers are generated from the progenitors of the neocortical germinal zone. In the adult brain, the different cortical layers are defined based on morphologically and functionally divergent neurons.

The six cortical layers are generated during embryonal development in a strictly regulated manner.

Following the formation of the cortical plate, the deeper layers neurons (including layers V and VI) and the upper layers neurons (layers II-IV) are generated.

Glial cells, including astrocytes and oligodendrocytes, are then generated at the latest stages of cortical development.

Neocortical neurogenesis depends on several transcriptional factors. For instance, LHX2 and PAX6 play a crucial role in the specification of neocortical progenitors, giving rise to the projection neurons. In addition, MEF2C is another transcription factor essential for normal neural development and spatial distribution in the neocortex.

Our selected markers for the cortical layers provide a useful tool for studying the development, the functional neuroanatomy and the pathology of the cerebral cortex.
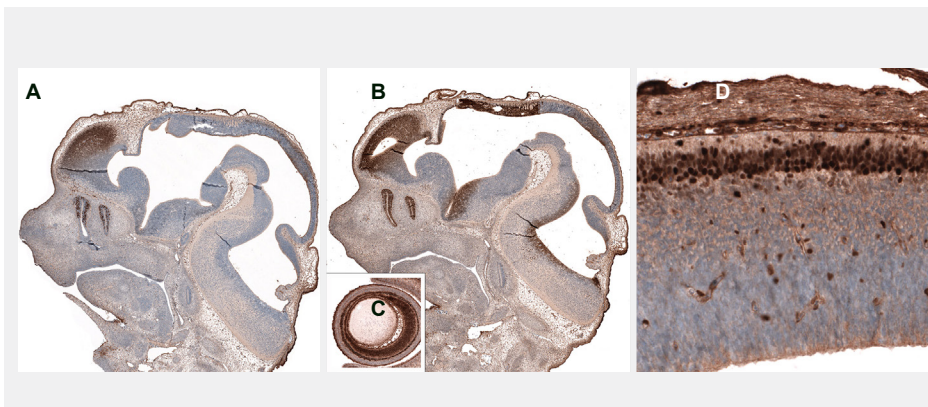
Figure 1. Tissue expression of transcription factors involved in the development of the cerebral cortex: LHX2, PAX6 and MEF2C.
A. Immunohistochemical staining with Anti-LHX2 antibody (AMAb91404) shows strong immunoreactivity in the developing cortex and sensory organs of mouse embryo E11.
B. PAX6 expression is detected in both the developing brain and sensory organs, including the eye (**C**) using the monoclonal antibody AMAb91372.
D. MEF2C is primarily expressed in the upper layers of the developing cortex, as shown by the anti-MEF2C antibody (AMAb90728) in mouse embryo E14.
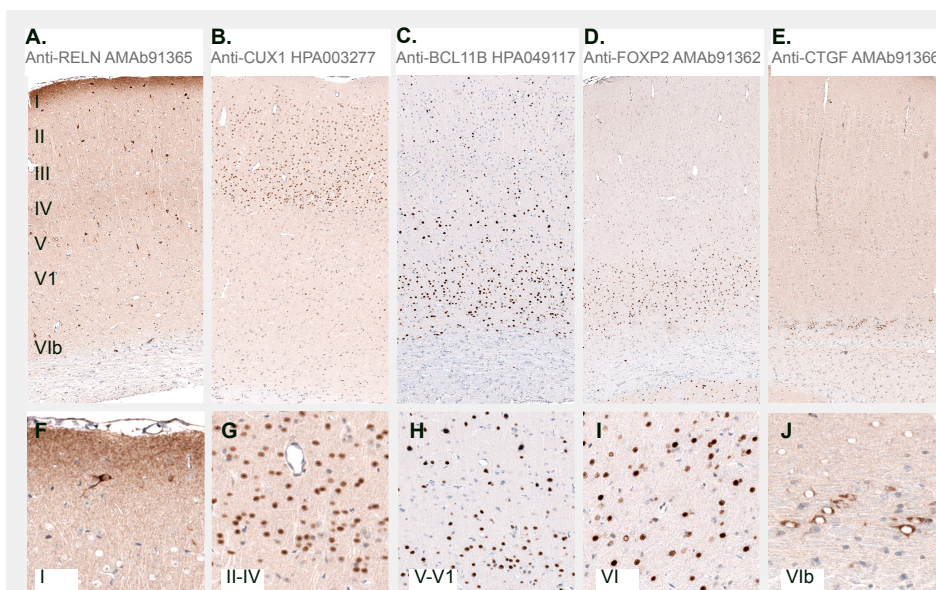
Figure 2. Laminar distribution of protein expression profiles in the cerebral cortex of rat.
A,F. RELN immunoreactivity is mainly detected in the neuropil of layer I (AMAb91365).
B,G. CUX1 expression is visible in layers II-IV (HPA003277).
C,H. BCL11B (CTIP2) is expressed primarily in layers V and VI (HPA049117).
D,I. Layer VI neurons show strong expression of FOXP2 (AMAb91362).
E,J. Layer VIb can be identified by the presence of CTGF immunoreactivity (AMAb91366).

Anatomic and functional diversity of neurons in the six cortical layers

In the mature neocortex, neurons of different layers display anatomical and functional diversity, including cell morphology, physiological properties, and anatomical connections.

Neurons of layers II/III, along with a subset of neurons of layer V, contribute mostly to intracortical connections, including the callosal projections to the contralateral cerebral hemisphere.

Layer V corticofugal neurons target the midbrain, hindbrain, and spinal cord, while layer VI neurons project mainly to the thalamus.

Protein expression profiles differ in neurons of various layers: for example, upper layers neurons can be identified by expression of CUX1 and POU3F2 (BRN2); the neurons of layer V express BCL11B (CTIP2), while neurons of layer VI express FOXP2. The laminar distribution of these and other markers in the human and rat cortex is shown in Figures 2-6.
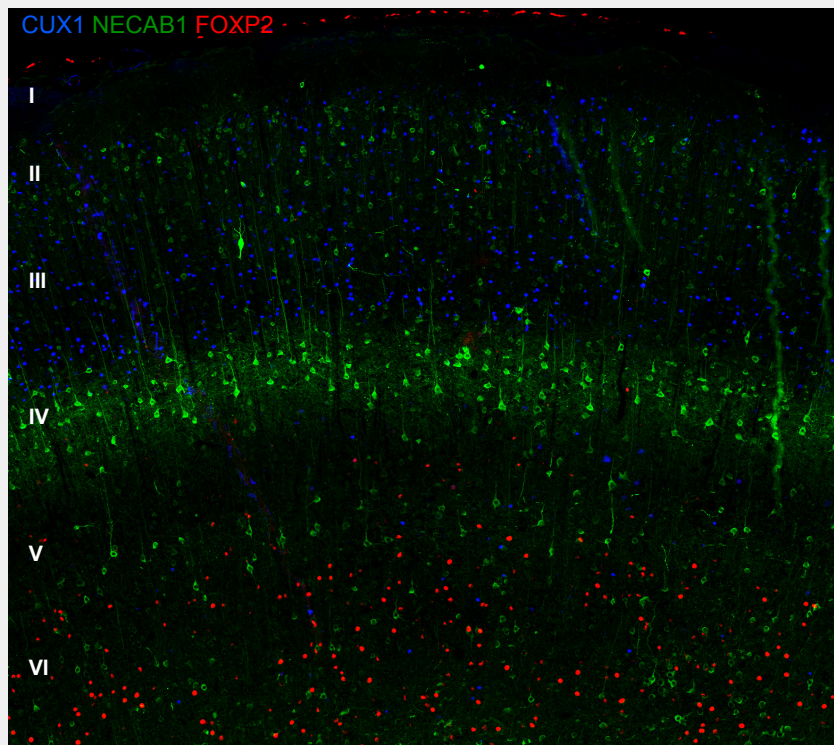
**Figure 3.** Laminar structure of the rat cerebral cortex.
Laminar structure of cerebral cortex of rat as shown by multiplexed immunofluorescence using the Anti-CUX1 (HPA003277, blue), the Anti-NECAB1 (AMAb90800, green), and the Anti-FOXP2 (AMAb91362, red) antibodies. Note that CUX1 is mainly expressed in layers II-III, NECAB1 shows the most robust expression in layer IV neurons, while FOXP2 is primarily present in layer VI neurons.

Table 1.
## Selected markers from Atlas Antibodies targeting cortical layers for tissue and cell analysis.

Cortical Layer	Product Name	Product number	Clonality	Validated Applications	Sequence Identity (Mouse/Rat)
Layer 1	Anti-RELN	AMAb91365	Monoclonal	IHC	90%/94%
Layer 1	Anti-RELN	HPA046512	Polyclonal	ICC-IF	90%/94%
Layer 2/3	Anti-RASGRF2	HPA018679	Polyclonal	IHC	72%/71%
Layer 2/3	Anti-CALB1	HPA023099	Polyclonal	IHC*, WB, ICC-IF	98%/99%
Layer 2/3-4	Anti-CUX1	AMAb91352	Monoclonal	IHC, ICC-IF	72%/74%
Layer 2/3-4	Anti-CUX1	AMAb91353	Monoclonal	IHC, ICC-IF	72%/74%
Layer 2/3-4	Anti-CUX1	HPA003277	Polyclonal	IHC	72%/74%
Layers 2/3, 4 and 5b	Anti-POU3F2 (BRN2)	HPA056261	Polyclonal	ICC-IF	100%/100%
Layers 2/3, 4 and 5b	Anti-POU3F2 (BRN2)	AMAb91406	Monoclonal	IHC, WB*, ICC-IF	100%/100%
Layers 2/3, 4 and 5b	Anti-POU3F2 (BRN2)	AMAb91407	Monoclonal	IHC, ICC-IF	100%/100%
Layer 2-4 (mainly 4)	Anti-NECAB1	AMAb90798	Monoclonal	IHC, WB	98%/98%
Layer 2-4 (mainly 4)	Anti-NECAB1	AMAb90800	Monoclonal	IHC, WB	98%/98%
Layer 2-4 (mainly 4)	Anti-NECAB1	AMAb90801	Monoclonal	IHC, WB	98%/98%
Layer 2-4 (mainly 4)	Anti-NECAB1	HPA023629	Polyclonal	IHC*, WB*	98%/98%
Layer 2-4 (mainly 4)	Anti-NECAB1	HPA031262	Polyclonal	IHC*, WB*	97%/98%
Layer 5	Anti-PCP4	HPA005792	Polyclonal	IHC*	96%/96%
Layer 5	Anti-CNTN6	HPA016645	Polyclonal	IHC	86%/84%
Layers 5-6	Anti-BCL11B (CTIP2)	HPA049117	Polyclonal	IHC*, ICC-IF	96%/98%
Layer 6	Anti-FOXP2	AMAb91361	Monoclonal	IHC, ICC-IF	99%/97%
Layer 6	Anti-FOXP2	AMAb91362	Monoclonal	IHC, ICC-IF	99%/97%
Layer 6	Anti-FOXP2	HPA000382	Polyclonal	IHC, WB	99%/97%
Layer 6	Anti-TLE4	HPA065357	Polyclonal	ICC-IF	100%/100%
Layer 6b	Anti-CTGF	AMAb91366	Monoclonal	IHC, WB	96%/97%
Layer 6b	Anti-CTGF	HPA031075	Polyclonal	IHC, WB, ICC-IF	96%/97%

* Products with enhanced validation for indicated applications
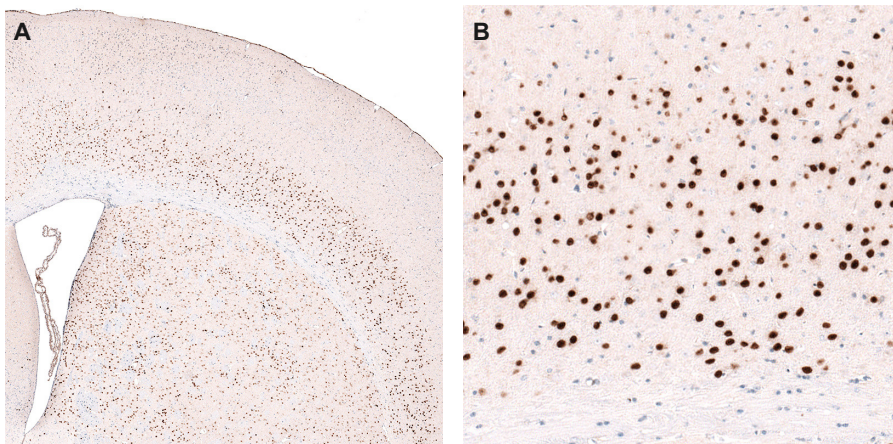
Figure 4.
A,B. Immunohistochemical staining of rat brain using the Anti-FOXP2 monoclonal antibody (AMAb91362) shows nuclear immunoreactivity in cortical layer 6 neurons, as well as in caudato-putamen, in brown.
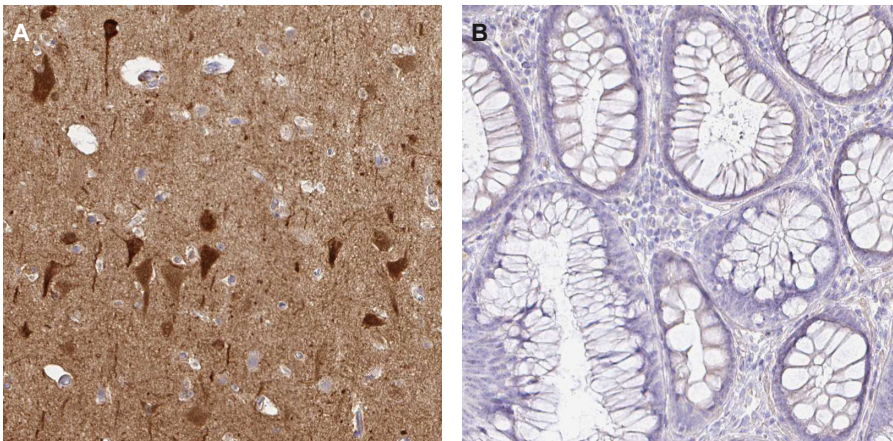
Figure 5.
A. Immunohistochemical staining of human cerebral cortex using the Anti-NECAB1 polyclonal antibody (HPA031262) shows strong cytoplasmic immunoreactivity in neurons, in brown, and no positivity in human colon, as expected (**B**).
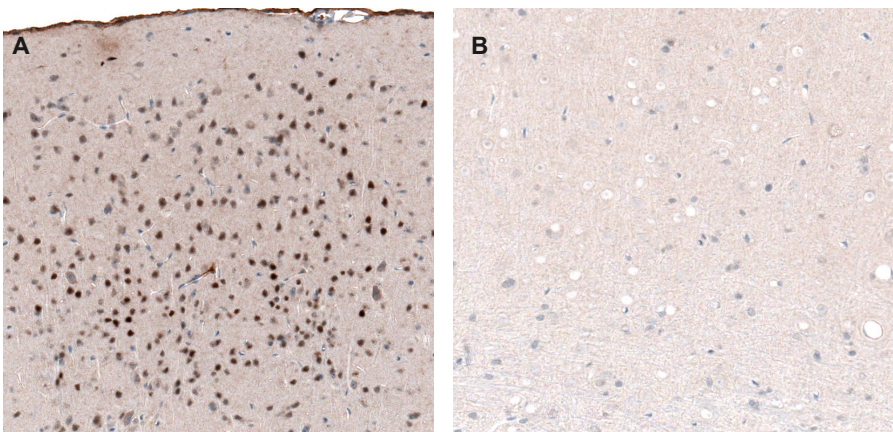
Figure 6.
A. Immunohistochemical staining of the mouse cerebral cortex using the Anti-POU3F2 monoclonal antibody (AMAb91407) shows moderate to strong nuclear positivity in neurons in layers 2-5, and no positivity in neurons of layer 6, as expected (**B**).

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