

Publication

A single protocol to detect transcripts of various types and expression levels in neural tissue and cultured cells: in situ hybridization using digoxigenin-labelled cRNA probes

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We have developed a simple non-radioactive in situ hybridization procedure for tissue sections and cultured cells using digoxigenin-labelled cRNA probes. This protocol can be applied for the detection of various transcripts present at a wide range of expression levels in the central nervous system. Cerebellar hybridization signals for transcripts estimated to be expressed at high (MBP, myelin basic protein), moderate (GluR1, subunit of AMPA/kainate sensitive glutamate receptors) and low (inositol polyphosphate-5-phosphatase) levels of abundance are demonstrated as examples. The sensitivity and cellular resolution were significantly improved by avoiding any ethanol treatment commonly used in other procedures. The localization of a labelled cell with respect to its environment is shown to be more easily assessed by counterstaining of the tissue with the nuclear dye Hoechst 33258. The present protocol can be combined with immunocytochemistry as demonstrated for glial fibrillary acidic protein (GFAP). All steps of the procedure, including preparation and labelling of the cRNA probes, pretreatment of tissue, hybridization and visualization of the labelled transcripts, are described in detail.

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