Application Note

Enhanced Detection of Kynurenine Aminotransferase II (KATII) in the Mouse Prefrontal Cortex Using MUSE® Signal Amplification

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Summary

In this project, MUSE® signal amplification technology (arcoris bio AG) was applied to improve the detection of the enzyme KATII in mouse prefrontal cortex sections. Compared to conventional secondary antibody-based immunohistochemistry (IHC), MUSE® amplification markedly enhanced the signal-to-background ratio and revealed a more restricted cellular expression pattern. KATII immunoreactivity was predominantly associated with astrocytes rather than neurons, consistent with previous findings in rat brain and gene expression data.[4] These results demonstrate that MUSE® amplification enables sensitive and specific detection of low-abundance proteins in mouse brain tissue while maintaining compatibility with standard immunohistochemistry workflows.

Introduction

Kynurenic acid (KYNA) is a key metabolite of the kynurenine pathway, and the only known endogenous antagonist of NMDA receptors in the brain.[1] Dysregulation of KYNA levels has been implicated in several neurological and psychiatric disorders, including Parkinson's disease and schizophrenia.[2,3] Kynurenine aminotransferase II (KATII) catalyzes the irreversible conversion of kynurenine to KYNA and is therefore a critical regulator of KYNA production in the central nervous system.[1] Previous studies have localized KATII primarily to astrocytes in the rat brain (see Fig. 1A).[4] However, attempts to reproduce this cell-specific expression pattern in mouse brain tissue using conventional fluorescent IHC have yielded widespread staining predominantly associated with neuronal populations (see Fig. 1B). This discrepancy suggests that conventional IHC may lack the sensitivity required to reliably detect endogenous KATII in the mouse PFC, potentially due to its low abundance and resulting low signal-to-background ratio. Signal amplification approaches offer a potential solution to this limitation. MUSE® (Multiplexed Universal Signal Enhancement) technology enables increased detection sensitivity while maintaining spatial resolution and compatibility with multiplex immunofluorescence. In this project, MUSE® amplification was applied to mouse prefrontal cortex sections to improve KATII detection. Using co-labeling with the neuronal marker NeuN and the astrocytic marker glutamine synthetase (GS), the cellular distribution of KATII was compared between a conventional IHC and a MUSE®-amplified staining.

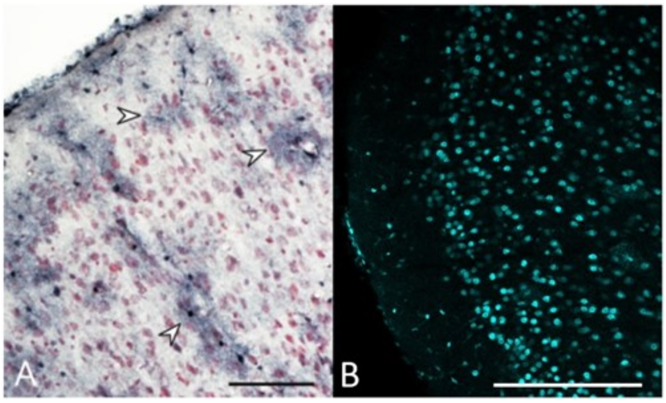


Figure 1: Comparison of KATII immunostaining in rat and mouse prefrontal cortex (PFC). A) KATII localization in predominantly astrocytes (blue, indicated by white arrows) in the rat PFC, combined with a neuronal staining (NeuN, red), modified from Guidetti et al., Glia, 2007. B) KATII localization (cyan) in the mouse PFC shows widespread expression predominantly in neuron-like cell bodies, using classical immunohistochemistry. Scale bars = 250 µm.

Methods

Tissue preparation: C57BL/6N mice (n = 10) were transcardially perfused with ice-cold 4% paraformaldehyde (PFA). Brains were extracted, post-fixed for 6 h in 4% PFA, and cryoprotected in 30% sucrose in PBS for 24–48 h. Coronal sections (30 µm) were cut using a sliding microtome and stored at −20 °C in cryoprotectant solution until further processing. Immunofluorescence staining: For immunofluorescent staining, free-floating sections were rinsed in Tris buffer (pH 7.4) and incubated in AB buffer provided by Arcoris Bio for 10 min at RT. Primary antibodies against NeuN (guinea pig, 1:1000, Synaptic Systems, 266 004), GS (mouse, 1:500, BD Transduction Lab, 610518), and KATII (rabbit, 1:1000, Proteintech, 13031-1-AP) were applied overnight at 4 °C in AB buffer containing 0.2% Triton X-100. Prior to staining, the KATII antibody was modified with an azide group using the Site-Click™ Antibody Azido Modification Kit (Invitrogen, S20026) according to the manufacturer's instructions. Following 3 x 10 min washes in Tris buffer and 2 x 2 min washes in 2X SSC buffer, sections were incubated with MUSE® A/B reagents (1:1) for 2 h at room temperature. Control sections were processed in parallel using Tris buffer. After 5 x 2 min washes in 2X SSC buffer, sections were incubated with MUSE® C/D reagents (1:1) containing secondary antibodies (donkey anti-mouse Cy3 1:500; donkey anti-guinea pig Cy5, 1:500), and DAPI (Thermo Fisher, 1:3000), protected from light. Control sections were incubated in Tris buffer containing the same antibodies, including an additional donkey anti-rabbit Alexa488 at 1:1000 instead. After 4 x 2 min washing in 2X SSC buffer, sections were mounted on gelatin-coated slides, coverslipped with fluorescence mounting medium (Agilent, S3023), and stored at 4 °C in the dark until image acquisition.

Image acquisition: Images were acquired using laser scanning confocal microscopy (Zeiss LSM800) and a 25× (oil, NA 0.8) objective.

Results

Conventional IHC revealed widespread KATII immunoreactivity throughout the mouse PFC, with near-complete co-localization with the neuronal marker NeuN and no detectable overlap with the astrocytic marker GS (see Fig. 2). This staining pattern suggested predominant neuronal expression of KATII and an absence of astrocytic localization, which contrasts with previous reports in rat and human brain.[1,4] However, prior gene expression analyses from our group indicated low levels of KATII expression in the mouse PFC, inconsistent with the broad distribution observed using conventional IHC (see Fig. 2). This discrepancy suggests that the apparent widespread signal may, at least in part, reflect a low signal-to-background ratio.

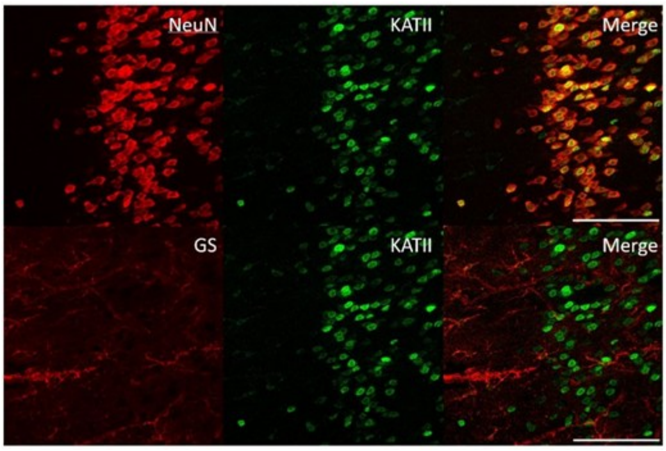


Figure 2: KATII localization in the mouse prefrontal cortex using classical immunohistochemistry. KATII detection using a secondary antibody conjugated to Alexa Fluor 488 (green) shows extensive co-localization with NeuN-positive neurons (red) and no detectable overlap with GS-positive astrocytes (red). Scale bars = 100 µm.

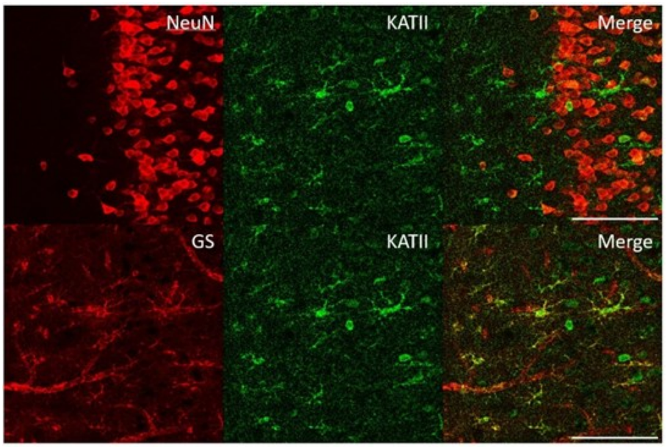
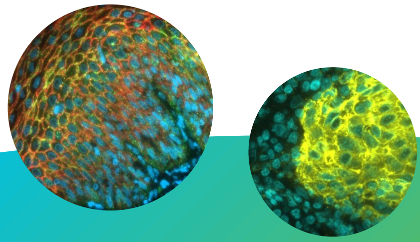


Figure 3: KATII localization in the mouse prefrontal cortex using MUSE® amplification. KATII detection using MUSE® amplification (FITC, green) reveals a more restricted expression pattern with predominant co-localization with GS-positive astrocytes (red) and minimal overlap with NeuN-positive neurons (red). Scale bars = 100 µm.



In contrast, MUSE® amplification resulted in a markedly improved signal-to-background ratio under identical imaging conditions. KATII immunoreactivity appeared more restricted and showed predominant co-localization with the astrocytic marker GS, while co-localization with NeuN-positive neurons was substantially reduced (see Fig. 3). This staining pattern is consistent with both gene expression data and previously reported cellular localization of KATII in the rat brain.[4] Together, these findings indicate that MUSE® amplification enhances detection sensitivity and improves KATII localization, revealing a cellular distribution that more accurately reflects its expected astrocyte-associated expression in the PFC.[4]

Conclusion

MUSE® signal amplification significantly improves the detection of low-abundance proteins such as KATII in brain tissue by enhancing signal intensity relative to background. The resulting staining pattern more accurately reflects the expected astrocyte-associated expression of KATII, consistent with observations in other species.[4] Importantly, this approach requires only minor modifications to standard immuno-histochemistry workflows and remains fully compatible with multiplex labeling strategies. MUSE® technology, therefore, represents a robust and versatile tool for cell-type-specific protein analysis and may enable more precise investigations of the kynurenine pathway in both physiological and disease contexts.

References

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