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Clearing of deparaffinized human brain tissue

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"Quantitative cytoarchitectural phenotyping of deparaffinized human brain tissues"

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Abstract

This protocol is a detailed version of the deparaffinization protocol for human brain tissue applicable to different clearing protocols (SHORT and iDISCO). The method was optimized by the European Laboratory for Non-Linear Spectroscopy - University of Florence -Italy, and describes the process to deparaffinize human brain tissue to prepare them for optical clearing and fluorescent labelling, permitting the 3D reconstruction of the tissue at micrometer-resolution using fluorescence microscopy techniques.

Attachments

PDF

final_draft.pdf
2.9MB

Deparaffinization of brain tissue

1 Deparaffinization was achieved by first removing as much paraffin surrounding the tissue block as possible with a blade. To completely remove the remaining paraffin, samples were placed in a water bath at 60°C for 30-60 min until the paraffin was macroscopically eliminated. In order to speed up this step and completely clean samples from the paraffin, tissue blocks were positioned on a porous membrane inside a 50 ml tube floating in the water bath. Following the paraffin melting, tissue blocks were incubated 6-8 times in 98% Xylene for 30 minutes at RT, using a rotary shaker. At this point, tissue blocks should appear translucent. Afterwards, samples were washed with 100%, 95%, and 70% ethanol diluted with water at RT for 30 min respectively, and further washed 3 times with 1× PBS for 10 min at RT. After embedding in 4% agarose, tissue sections of 500 ± 50 µm-thick were obtained using a vibratome (Leica VT1000 S) and stored at 4°C in PBS + 0.01% w/v NaN_3.

Application to SHORT tissue transformation method

2 Single deparaffinized slices with a thickness of 500 µm were processed with the SHORT tissue transformation method, as previously described. The specimens were first incubated in a Switch-Off solution (50% v/v 1× PBS (pH 3), 25% v/v 0.1 M hydrochloric acid (HCl), 25% v/v 0.1 M potassium hydrogen phthalate (KHP), and fresh 4% v/v glutaraldehyde) at 4°C in gentle shaking for 24 h. The solution was replaced with the Switch-On (1× PBS (pH 7.4) and fresh 1% v/v glutaraldehyde) and the incubation was performed at 4°C in gentle shaking for 24h. Afterwards, slices were washed 3 times for 2 h each in 1× PBS at RT and reactive glutaraldehyde was inactivated by o/n (overnight) incubation with an inactivation solution (1× PBS (pH 7.4), 4% w/v acetamide, 4% w/v glycine, pH 9.0) at 37°C in a water bath. Following inactivation, the samples were washed in PBS at RT and then incubated in the clearing solution (200 mM sodium dodecyl sulfate (SDS), 20 mM sodium sulfite (Na_2SO_3), and 20 mM boric acid (H_3BO_3), pH 9.0) for 3-6 days at 55°C. After the clearing step, samples were extensively washed in 1× PBS + 0.1% Triton X-100 (PBST) at 37°C for 24 h. Next, the slices were treated with 30% H_2O_2 for 45 min and antigens were unmasked with the preheated antigen retrieval solution (10 mM Tris base (v/v), 1 mM EDTA solution (w/v), 0.05% Tween 20 (v/v), pH 9) for 10 min at 95°C. After cooling down to RT, the specimens were washed in deionized (DI) water for 5 min each and then equilibrated with PBS for 1 h. The primary antibodies were diluted in PBST + 0.01% w/v NaN_3 , and sample incubation was performed at 37°C in gentle shaking. After washing with PBST at 37°C for 24 h, the stained samples were incubated in gentle shaking with the secondary antibodies at 37°C in PBST + 0.01%

w/v NaN_3. Next, the stained samples were extensively washed with PBST at 37°C, equilibrated first in 30% TDE/PBS (v/v), then in 68% TDE/PBS (v/v), and finally placed in the sandwich with 68% TDE.

Application to iDISCO method

3 Single deparaffinized slices with a thickness of 500 µm were processed with a modified iDISCO protocol. Specimens were dehydrated with increasing concentrations of methanol MeOH/H_2O (20%, 40%, 60%, 80%, and 100%), with gentle rotation for 1 h each at RT. Samples were then incubated in 66% dichloromethane (DCM) / 33% MeOH overnight at RT with gentle rotation. Afterwards, samples were washed with 100% MeOH for 1 h twice, and autofluorescence was bleached through incubation with 5% H_2O_2 in MeOH at 4°C overnight, without shaking. After bleaching, samples were rehydrated with decreasing concentrations of MeOH/H_2O (80%, 60%, 40%, 20%), followed by one PBS and two PBST (1× PBS and 0.2% v/v Triton X-100) washes, all with gentle rotation for 1 h each at RT. On the same day, samples were incubated first with the Permeabilization solution (1× PBS, 0.2% v/v Triton X-100, 20% v/v DMSO and 0.3M glycine), followed by incubation in Blocking solution (1× PBS, 0.2% v/v Triton X-100, 0.2% w/v gelatin porcin skin and 0.01% w/v NaN_3), both for 24 h, at 37°C with gentle rotation. Primary antibodies were diluted in PBSTW-Heparin (1× PBS, 0.2% v/v TWEEN-20 and 0.01 mg/ml Heparin) for 6 days, at 37°C with gentle rotation. Samples were then washed for 1 day in PBSTw-Heparin and then incubated with the secondary antibodies in PBSTw-Heparin for 4 days, at 37°C with gentle rotation. After 1 day of washes in PBSTw-Heparin, samples were dehydrated as previously described and incubated for 3 h in 66% DCM / 33% MeOH. Finally, they were washed in 100% DCM for 30 min twice and incubated and stored in DBE (Dibenzyl ether) at RT.

Protocol references

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