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Protocol status: Working
We use this protocol and it's working

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DAPI Staining Mouse Brain Sections

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ABSTRACT

This protocol details the steps for staining 4% PFA fixed mouse brain tissue sections with DAPI (4',6-diamidino-2-phenylindole), a fluorescent stain that can be used to label anatomic regions of interest in a mouse brain, allowing for imaging with a fluorescent microscope. The protocol includes a table with suggested staining duration based on section thickness and guidelines for calculating the volume of DAPI solution needed for staining.

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MATERIALS

DAPI **Thermo Fisher Scientific Catalog #**D1306 ([M] 5 mg/mL)

Phosphate Buffered Saline **Thermo Fisher Scientific Catalog #**28374

Sodium azide **P212121**

	Materials	Product number
	Aluminum foil	Amazon, B074NB5CDZ
	48 well plate or equivalent staining plate	Costar, 3548
	Corning 50ml centrifuge tube	Millipore Sigma, CLS430829
	15ml centrifuge tube	Globe Scientific, 6264
	Benchmark vortex mixer	Southern Labware, BV1000
	Manual single channel pipettes: P20, P200, P1000	Rainin, 30456871
	Manual single channel pipette: P2	Rainin, 17014393
	Manual single channel pipettes: P5000	Rainin, 17011790
	Nutating mixer	Fisherbrand, 88-861-043
	Stir bar	Grainger, 21R590

1L 1xPBS:

Combine the following reagents into a container with a stir bar. A graduated cylinder may be used to measure MilliQ water and a graduated cylinder or serological pipet may be used to measure 10xPBS. Mix well on a stir plate at high speed (300 RPM or higher) for 00:02:00 or until solution is mixed. Store at Room temperature for 1 month.

	Reagent	Volume
	Milli-Q water	900mL
	10xPBS	100mL

1L 1xPBS & 0.01% Sodium Azide:

Combine the following reagents into a container with a stir bar, using a graduated cylinder to measure the 1xPBS and a P5000 pipette to measure the 0.01% sodium azide. Mix well on a stir plate at high speed (300 RPM or higher) for 00:02:00 or until solution is mixed. Store at Room temperature or 4 °C for up to 1 year.

	Reagent	Volume
	1xPBS	999mL
	Sodium Azide	1mL

2mL 5mg/mL DAPI solution:

Add Milli-Q water to DAPI powder in 10mg vial using P5000 pipette. Vortex until powder completely mixes into solution. Store at 4 °C and vortex before use.

	Reagent	Volume
	Milli-Q water	2mL
	DAPI powder	10mg

SAFETY WARNINGS

DAPI is a mutagen and should be handled with care. Wear PPE and dispose into hazardous waste stream. Please consult your immediate supervisor or the EH&S manager/representative if you have questions or concerns.

Sodium Azide is toxic and carcinogenic. It should be handled and prepared with care. Do not breathe dust, do not use metal utensils. Wear gloves when handling this chemical.

Paraformaldehyde is carcinogenic. Wear gloves at all times when handling specimen fixed in paraformaldehyde, as the tissue may contain trace amounts of the chemical.

BEFORE START INSTRUCTIONS

This protocol details DAPI staining for 4% PFA fixed mouse brain tissue that has already been sliced. Refer to protocol Sectioning Mouse Brain with Sliding Microtome for information about preparing slices ahead of DAPI staining.

Throughout this protocol, tissue should be protected from light by covering well plate with aluminum foil between steps or equivalent protective measure.

Before Staining

1 Wash the mouse brain sections for at least 3 times, 00:05:00 each time in 1XPBS. 5m

Safety information

Paraformaldehyde is carcinogenic. Wear gloves at all times when handling specimen fixed in paraformaldehyde, as the tissue may contain trace amounts of the chemical.

Diluting DAPI

2

Dilute 5mg/mL DAPI solution to 1:5000 with 1xPBS by measuring 1XPBS into a conical tube and adding DAPI with a manual pipette. Vortex DAPI before pipetting into 1XPBS.

Safety information

DAPI is a mutagen and should be handled with care. Wear PPE and dispose into hazardous waste stream. Please consult your immediate supervisor or the EH&S manager/representative if you have questions or concerns.

Note

Volume will vary based on the amount of solution needed. For a standard 48 well plate, 200uL of DAPI solution should be prepared for each well in order to fully submerge the tissue in solution, and this may be used to calculate the total volume of solution needed.

If staining in a plate with larger wells (ex: 24 well plate, 12 well plate, or Netwell cell culture inserts), a larger volume of DAPI solution will be needed in order to fully submerge the tissue in solution, and this should be used to calculate the total volume of solution needed.

Example recipe for 5mL total volume DAPI:1xPBS solution:

Reagent	Volume
1xPBS	5mL
DAPI	1uL

Staining with DAPI

50m

3 Pipette diluted DAPI (see note below) into each well with a manual pipette and place well plate on a shaker at Room temperature. Each well should contain enough DAPI solution to fully submerge the sections within. Leave well plate on shaker for suggested staining duration based on section thickness (see table below). Ensure that the well plate is covered in aluminum foil and away from direct light.

Section thickness	Staining duration
50 microns	15 minutes
100 microns	30 minutes

Safety information

Sodium Azide is toxic and carcinogenic. It should be handled and prepared with care. Do not breathe dust, do not use metal utensils. Wear gloves when handling this chemical.

Note

200uL is sufficient volume to full submerge tissue in diluted DAPI solution in each well in a standard 48 well plate. If staining in plates with larger wells (ex: 24 plate well, 12 plate well), a larger volume of solution may be needed to fully submerge the tissue in each well.

4 After the incubation period, wash sections at least **5 times** at 00:05:00 each with 1xPBS. Refer to protocol Mounting and Coverslipping Mouse Brain Sections for next steps. 25m

5 **If unable to mount on the same day:**
After 1X PBS washes, place sections in 1xPBS & 0.01% sodium azide solution, cover well plate with aluminum foil to protect from light, and store in 4 °C until ready for mounting.