Immunofluorescence staining (cells and tissues)

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- Antigen Retrieval Buffer
  - 10mM Tri-Soduim Citrate (dihydrate)
  - 5mM EDTA
  - 0,05% Tween 20
  - pH 6,0
- Xylene
- Ethanol
  - 100% / 95% / 70% / 50%
- PBS
  - 140 mM NaCl
  - 0,27 mM KCl
  - 0,15 mM K_2HPO_4
  - 7,1 mM Na_2HPO_4 x $2H_2O$
  - pH 7,2-7,4
- IF buffer
- 10mM ethanolamine. in PBS
- NABH4 (1%)
- Aurion BSA (1%) = Blocking Buffer
- Donkey Serum/ Sheep Serum
- Liquid Blocker Pen
- Fluorescene mounting media

Procedure

1st Day: removing Parafin, rehydrate, ... and incubate 1st antibody

✔ 1. Rinse cells or tissue in PBS briefly to remove media components. **(cells only)**

- ✔ 2. Fix cells or tissue in 4% paraformaldehyde in PBS (freshly prepared) for 15 min at 4ºC. **(cells only)**
- ✔ 3. Prepare 10% para. stock like this: 1g paraformaldehyde in 10 ml dH_2O Heat to ~60°C Add 1 drop of 1M NaOH and continue heating until most is dissolved. Filter stock thru 0.2 µm filter **(cells only)**
- ✔ 4. For removing the Parafin use Xylene for Rehydration use different concentrations of Ethanol procede the Following Protocol **(Tissues only)**

15 min	Xylene
15 min	Xylene
15 min	Xylene
5 min	Ethanol 100%
5 min	Ethanol 100%
5 min	Ethanol 95%
5 min	Ethanol 70%
5 min	Ethanol 50%
5 min	PBS

- ✔ 5. While Samples are in the PBS prepare Antigen retrieval Buffer and Heat the Presure cooker up to 120°C **(Tissues only)**

  Boil the Samples under Pressure for 30 minutes and afterwards let them cool to room tempereature.
- ✔ 6. Mark your Samples with the Liquid Blocker Pen by drawing circles around them
- ✔ 7. Incubate with 10mM ethanolamine for 5 min at RT, if needed
- ✔ 8. Wash 3X 1 min with PBS
- ✔ 9. Incubate with 0.1% Triton-X100 in PBS for 1 min at RT
- ✔ 10. Wash 3X 1 min with PBS
- ✔ 11. Incubate the Samples with 1% NABH4 in PBS , to deminish backgroundby dropping into the circles for 20 minutes (perhaps Liquid Blocker Pen circles need to be drawn again) (only for tissue)
- ✔ 12. Wash the samples 3 times with IF-buffer for 5 minutes
- ✔ 13. Block the autoactivity of the Tissue with Aurion 1% Aurion BSA, 1% Serum (Depending on source of secondary antibody) (Blocking Buffer) in IF-bufferfor 1 hour
- ✔ 14. Dilute multiple 1st Antibody (usualy 1:100) in Blocking Buffer and incubate overnight at 4°C

2nd Day

- ✔ 15. Wash the Slides 3 times with IF-buffer for 15 minutes
- ✔ 16. Incubate with the correspondinfg 2nd Antibodies diluted in Blocking Buffer for 1 hour at RT
- ✔ 17. Wash the Slides 3 times with IF-buffer for 15 minutes in dark.

- ✔ 18. Incubate 5 min with 2X DAPI in PBS in dark.
- ✔ 19. Wash the Slides 1 time with IF-buffer for 5 minutes in dark.
- ✔ 20. Mount the coverslip using Fluorescence mounting media.