🌐 cFos DAB IHC Protocol

Yueyi Chen[1], Adam Kimbrough[1], Olivier George[1]

[1]University of California, San Diego

Upload image

Jul 23, 2020

1 *Works for me*   This protocol is published without a DOI.

George Lab
Tech. support email: **olgeorge@ucsd.edu**

Yueyi Chen

PROTOCOL CITATION

LICENSE

CREATED

Nov 06, 2019

LAST MODIFIED

Jul 23, 2020

PROTOCOL INTEGER ID

29534

MATERIALS TEXT

cell signaling cFos Rabbit Monoclonal #2250
Rabbit ImmPress Kit (Vector)
DAB substrate Kit (Vector) with NI
PBS
Tween 20
Hydrogen peroxide
normal donkey serum

1

Day 1

1.1   Rinse in PBS (if sections have been stored in PBS/Azide)

1.2   Quench endogenous peroxidase activity:
Incubate sections for 20 minutes in 1% Hydrogen peroxide/ PBS (330µl 30% H_2O_2 in 10 ml PBS)

1.3   Rinse in PBS 3 times x 10 minutes each

1.4   Blocking:

Incubate section at room temperature for a minimum of 60 minutes in PBS/ TX100 (0.3%), BSA (1 mg/ml) and 5% normal donkey serum (Jackson)

1.5 Antibody incubation:
Fos 1:1000 cell signaling cFos Rabbit Monoclonal #2250
Primary antibody diluent
PBS 0.5%, TWEEN 20, 5% normal donkey serum

1.6 Incubate primary antibodies 24 hours at RT degrees with agitation

2 Day 2

2.1 Rinse in PBS 3 times x 10 minutes each

2.2 Rabbit ImmPress reagent undiluted 1-2 hours

2.3 Rinse in PBS 3 times x 10 minutes

2.4 For cFos DAB NI
Incubate in Vector DAB substrate kit per kit instructions
For 10 mis use: 4dBuffer, 8dDAB, 4dH2O2, 4d NI

2.5 check reaction after 2 minutes to reduce background

2.6 check full development of cFos and note incubation time

2.7 rinse PBS 3 times x 10 minutes

2.8 mount on to coated slides
label experiment date, animal # and code, section #, cFos