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# Immunohistochemistry (immunostaining cultured rat hippocampal neurons) from Yao CK et al. (2017)

Chi-Kuang Yao, Yu-Tzu Liu, I-Chi Lee, You-Tung Wang, Ping-Yen Wu

## **Abstract**

This protocol describes the immunostaining of cultured rat hippocampal neurons to stain for ratFwe2 in cell body. It is from 'Flower Ca<sup>2+</sup> channel in CME and ADBE' of Yao CK et al.

Please see the manuscript for the full method details.

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#### **Guidelines**

To stain for Synaptophysin and mFwe2-HA in presynaptic compartments, the cells were permeabilized with 100% methanol at -20°C for 15 min. 1XPBS solution was used for all staining steps. Primary antibody dilutions used: rabbit anti-GFP, 1:500 (Invitrogen); guinea pig ant-m/ratFwe2 (GP67), 1: 100. rabbit anti-GM130, 1:500 (Adcam); mouse anti-HA, 1:200 (Sigma); mouse anti-SVP38, 1:200 (Sigma). Secondary antibodies were diluted in 1:500 (Jackson ImmunoResearch and Invitrogen). DAPI was used in 1: 2000 (Invitrogen). Images processing was achieved using LSM Zen.

#### **Materials**

- ✓ 1XPBS solution by Contributed by users
- √ 4% paraformaldehyde/4% sucrose/1XPBS solution by Contributed by users
- √ 10% BSA/0.1% Tween-20-containing 1XPBS solution by Contributed by users
- ✓ 0.1% Tween-20-containing 1XPBS solution by Contributed by users.
- 100% methanol by Contributed by users

#### **Protocol**

## Immunostaining cultured rat hippocampal neurons

## Step 1.

To stain for ratFwe2 in cell body, rinse DIV14 neurons cultured in 12-well plate with 1XPBS solution.

## Immunostaining cultured rat hippocampal neurons

## Step 2.

Fix them in 4% paraformaldehyde/4% sucrose/1XPBS solution for 10 min at room temperature.

© DURATION

00:10:00

## Immunostaining cultured rat hippocampal neurons

## Step 3.

Permeabilize the cells with 0.1% Tween-20-containing 1XPBS solution for 20 min at room temperature on a horizontal shaker. (1/3)

© DURATION

00:20:00

#### Immunostaining cultured rat hippocampal neurons

#### Step 4.

Permeabilize the cells with 0.1% Tween-20-containing 1XPBS solution for 20 min at room temperature on a horizontal shaker. (2/3)

**O** DURATION

00:20:00

## Immunostaining cultured rat hippocampal neurons

## Step 5.

Permeabilize the cells with 0.1% Tween-20-containing 1XPBS solution for 20 min at room temperature on a horizontal shaker. (3/3)

**O DURATION** 

00:20:00

## Immunostaining cultured rat hippocampal neurons

## Step 6.

Block further with 10% BSA/0.1% Tween-20-containing 1XPBS solution for 1 h.

**O** DURATION

01:00:00

#### Immunostaining cultured rat hippocampal neurons

## Step 7.

To reduce non-specific staining, absorb anti-m/ratFwe2 antibodies with paraformaldehyde-fixed n2a cells before staining.

#### Immunostaining cultured rat hippocampal neurons

#### Step 8.

Dilute primary antibodies in 0.1% Tween-20-containing 1XPBS solution.

## Immunostaining cultured rat hippocampal neurons

#### Step 9.

Incubate the cells with this staining solution at least for 8 h at 4°C.

© DURATION

08:00:00

## Immunostaining cultured rat hippocampal neurons

#### Step 10.

Wash the cells with 0.1% Tween-20-containing 1XPBS solution for 5 min at room temperature on a rotator. (1/3)

**O DURATION** 

00:05:00

## Immunostaining cultured rat hippocampal neurons

## **Step 11.**

Wash the cells with 0.1% Tween-20-containing 1XPBS solution for 5 min at room temperature on a rotator. (2/3)

**O DURATION** 

00:05:00

## Immunostaining cultured rat hippocampal neurons

## **Step 12.**

Wash the cells with 0.1% Tween-20-containing 1XPBS solution for 5 min at room temperature on a rotator. (3/3)

**O DURATION** 

00:05:00

## Immunostaining cultured rat hippocampal neurons

## **Step 13.**

Dilute secondary antibodies in 0.1% Tween-20-containing 1XPBS solution.

## Immunostaining cultured rat hippocampal neurons

## Step 14.

Incubate the cells with this staining solution at least for 2 h at room temperature on a rotator.

**O DURATION** 

02:00:00

# Immunostaining cultured rat hippocampal neurons

## **Step 15.**

Wash the cells with 0.1% Tween-20-containing 1XPBS solution for 5 min at room temperature on a rotator. (1/3)

**O** DURATION

00:05:00

## Immunostaining cultured rat hippocampal neurons

#### **Step 16.**

Wash the cells with 0.1% Tween-20-containing 1XPBS solution for 5 min at room temperature on a rotator. (2/3)

**O DURATION** 

00:05:00

## Immunostaining cultured rat hippocampal neurons

## **Step 17.**

Wash the cells with 0.1% Tween-20-containing 1XPBS solution for 5 min at room temperature on a rotator. (3/3)

**O DURATION** 

00:05:00

## Immunostaining cultured rat hippocampal neurons

#### **Step 18.**

Replace the wash solution with glycerol-containing mounting medium.

## Immunostaining cultured rat hippocampal neurons

## Step 19.

Mount the cells were further with glycerol-containing mounting medium.