

NB100-317 Protocol

Immunocytochemistry/Immunofluorescence Protocol for Telomerase reverse transcriptase Antibody (NB100-317)

TERT Antibody (2C4): https://www.novusbio.com/products/tert-antibody-2c4_nb100-317
Immunofluorescence

1. Cell growth and feeding for IF
 - A. Seed cells in 4-chamber slides at 20,000 per chamber.
 - B. Grow to medium confluence
 - C. Feed with MCDB170+IP at -48 and -24 hr.

2. Fixing cells for IF
 - A. Wash cells (~70-80% confluent) with 1XPBS
 - B. Fix slides each in 1:1 ice cold MEOH:acetone and place at -20C for 10 minutes.
 - C. Store no more than 48 hr in 100% ethanol.

3. IF for hTERT
 - A. Remove fixative/ethanol from slides.
 - B. Add 1 ml 2N HCl to each chamber.
 - C. Incubate for 20 minutes.
 - D. Remove the HCl and neutralize with 1 ml 0.1 M Na-borate.
 - E. Incubate for 5 minutes.
 - F. Remove Na-borate and add 1 ml blocking buffer.
 - G. Incubate for 2 hr at RT.
 - H. Prepare NB 100-297 at indicated dilution.
 - I. Incubate ON at 4C.
 - J. Wash 4X5 min. in RT PBS.
 - K. Add secondary (FITC conjugated rabbit anti-mouse IgM).
 - L. Incubate at RT for 2 hrs.
 - M. Wash 4X5 min. in 1X PBS.
 - N. Wash 5 min in 1X PBS with DAPI (1.5 ug/ml).
 - O. Rinse slides briefly on PBS.
 - P. Remove chambers from slides.
 - Q. Mount in Vectashield (Vector catalog # H1200) and observe.

Blocking buffer To 500 ml of 1X PBS:

- A. 5 g fish gelatin (Sigma catalog #G7765)
- B. 25 ml goat serum
- C. 5 g BSA Filter through 0.2 u filter and store at 4C