

PROTOCOL-AT-A-GLANCE

CELL CULTURE (24-well plate)

**WASH 1**

Remove cell culture medium and wash

1 mL

x 1

⌚ 1min

🌡️ 20-25°C

**1. FIXATION**2.5 µL FIX-REAG (200X)
+ 497.5 µL FIX-BUFF. 1X

0.5 mL

x 1

⌚ 10min

🌡️ 20-25°C

**WASH 1**40 µL Wash 1 (25X)
+ 960 µL dH₂O

1 mL

x 3

⌚ 3min

🌡️ 20-25°C

**2. PERMEABILIZATION**50 µL PERM-SOLN (10X)
+ 450 µL Wash 1

0.5 mL

x 1

⌚ 10min

🌡️ 20-25°C

(Slow agitation)

**WASH 1**40 µL Wash 1 (25X)
+ 960 µL dH₂O

1 mL

x 3

⌚ 3min

🌡️ 20-25°C

(agitation)

**3. STABILIZATION**

STAB-REAG + STAB-BUFF

0.5 mL

x 1

⌚ 10min

🌡️ 20-25°C

**WASH 1**40 µL Wash 1 (25X)
+ 960 µL dH₂O

1 mL

x 3

⌚ 3min

🌡️ 20-25°C

(agitation)

**4. SATURATION**

Ready to use

0.5 mL

x 1

⌚ 30min

🌡️ 20-25°C

(Slow agitation)

**5. PRIMARY ANTIBODY**2 µL Primary antibody
+ 998 µL AB-DILUT

0.5 mL

x 1

⌚ O / N

🌡️ 2-8°C

(Slow agitation)

**WASH 2**40 µL Wash 2 (25X)
+ 960 µL dH₂O

1 mL

x 3

⌚ 10min

🌡️ 20-25°C

(agitation)

**6. SECONDARY ANTIBODY**Secondary antibody
not provided

0.5 mL

x1

⌚ 1hour

🌡️ 20-25°C

(Protect from light)

**WASH 2**40 µL Wash 2 (25X)
+ 960 µL dH₂O

1 mL

x 3

⌚ 3 min

🌡️ 20-25°C

(Agitation - Protect from light)

**WASH 3**40 µL Wash 3 (25X)
+ 960 µL dH₂O

1 mL

x 3

⌚ 3min

🌡️ 20-25°C

(Agitation - Protect from light)

**7. MOUNTING & IMAGING**Mounting media
not provided