Protocol for Ki67 Antibody (Cat. No. 398 003) Immunohistochemistry Paraffin embedded (IHC-P) Fluorescence Staining

Tissue Fixation and Section Thickness

- 3.7% formaldehyde (24 h), 3.5 µM paraffin sections on superfrost slides

Materials and reagents

- **Food Steamer** (e.g. Braun, Multigourmet)
- Staining Containers with slide holders (e.g. Tissue-Tek)
- **Protein Block, Serum-Free** (Agilent X0909)
- **Antibody diluent** (Agilent S2022)
- **Primary antibody:** Ki67 antibody (cat. no. 398 003)
- **Secondary antibody:** Fluorophore conjugated rabbit specific secondary antibody
- **PBS:** Phosphate buffered saline (200 mM NaCl, 2,5 mM KCl , 8 mM Na_2HPO_4 , 1,5 mM KH_2PO_4), pH 7.4
- **Antigen Retrieval buffer**
  - **Citrate Buffer** (10 mM Citrate, 0.05% Tween 20, pH 6.0)
- **Deparaffinize and re/de-hydrate solution:** Xylene, 100% ethanol, 90% ethanol, 80% ethanol and 70% ethanol, 2-propanol
- **Optional:** DAPI (Roth 6335.1) (4 mg/ml in dH_2O)
- **Mounting medium:** glycerol-based, hard-setting, ready-to-use mountant
- **Coverslip**

Procedure

1. **Deparaffinize and hydrate tissue sections**
   - Xylol 2 x 5 min
   - 100% EtOH 2 x 2 min
   - 90% EtOH 1 x 2 min
   - 80% EtOH 1 x 2 min
   - 70% EtOH 2 x 2 min
   - Deionized Water 1 x 20 sec
   - PBS 1 x 2 min
     - Keep the slides in PBS until ready to perform the Antigen Retrieval.
     - Do not allow the slides to dry out.

2. Performe **Antigen Retrieval (AR) using a food steamer.**
   a. Heat the steamer with a suitable staining container filled with Antigen Retrieval buffer to **~97°C**
      - **Citrate Buffer** (10 mM Citrate, 0.05% Tween 20, pH 6.0)

b. Transfer the sections into the staining box, wait until the temperature reaches **97°C.**

c. Incubate the sections in the steamer for **30 min.**

d. Remove the staining container from the steamer and allow the slides to cool down for **20 min** (target end temperature **~60°C**).

3. Wash slides in PBS, 3 x 1 min.

4. Blocking non-specific binding in **Protein Block, Serum-Free** for **10 min.**

5. Drain slides (do not rinse).

6. Apply **primary antibody** diluted in **Antibody Diluent** and incubate in a humidified chamber **overnight at 4°C.**
   - Suggested dilution: **1:100** in **Antibody Diluent**

7. Wash slides in PBS, 3 x 2 min.

8. Apply **secondary antibody** diluted in **PBS for 60 min at room temperature.**
   - **Suggested concentration:** 2.5 µg/ml
   - *Note: Avoid bright light when working with the secondary antibody to minimize photo bleaching of the fluorescent dye.*

9. Wash slides in PBS, 3 x 2 min.
   - *Optional: Counterstain: Dilute DAPI stock solution 1:10,000 in PBS and apply for 5 min*

10. Wash slides in PBS, 2 x 1 min.

11. Wash slides in deionized water for 1 min.

12. Mount slides in a suitable **mounting medium** and add a **coverslip.**

Note: This protocol has been validated in the SYSY Antibodies laboratories to ensure consistent and reliable staining results. However, for achieving the best specific signal with minimal background, the optimal antibody concentration, incubation temperature, and incubation duration should be optimized for each experiment.