Bio'Ace BIOLOGICAL TECHNOLOGY
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Basic Information

Product Name	Anti-Ki67 Antibody (Clone#ST50-01)
Gene Name	Ki67
Source	Rabbit
Clonality	Monoclonal
Isotype	IgG
Species Reactivity	human
Tested Application	WB, IF-Cell, IHC-P, FC
Contents	1ug/ul in 1×TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Immunogen	Synthetic peptide within human Ki67
Concentration	1ug/ul
Purification	Protein A affinity purified
Observed MW	Predicted band size: 359 kDa

Recommended Dilution Ratios

Application	Dilution Ratios
WB	1:1,000-1:2,000
IF-Tissue	1:100
IF-Cell	1:100-1:1,000
IHC-P	1:100-1:500
FC	1:50-1:100

Storage

Store at +4°C after thawing. Aliquot store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

Background Information

Ki-67 is a nuclear protein that is expressed in proliferating cells and may be required for maintaining cell proliferation. Ki-67 has been used as a marker for cell proliferation of solid tumors and some hematological malignancies. A correlation has been demonstrated between Ki-67 index and the histopathological grade of neoplasms. Assessment of Ki-67 expression in renal and ureter tumors shows a correlation between tumor proliferation and disease progression, thus making it possible to differentiate high-risk patients. Ki-67 expression may also prove to be important for distinguishing between malignant and benign peripheral nerve sheath tumors.

Selected Validation Data

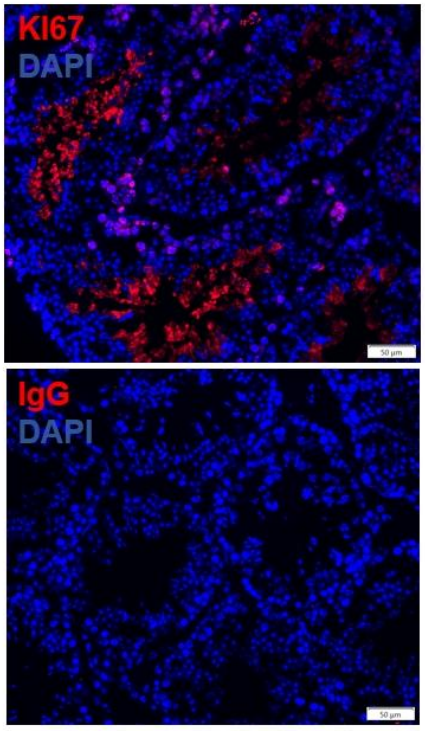

Figure 1. IF analysis of Ki67 using anti- Ki67 antibody (BA0007) in mouse testis. Ki67 was detected in a paraffin-embedded section of mouse testis tissue. 568 cy3 conjugated Anti- Rabbit IgG Secondary Antibody (Cyan) was used as secondary antibody. The section was counterstained with DAPI (Blue).

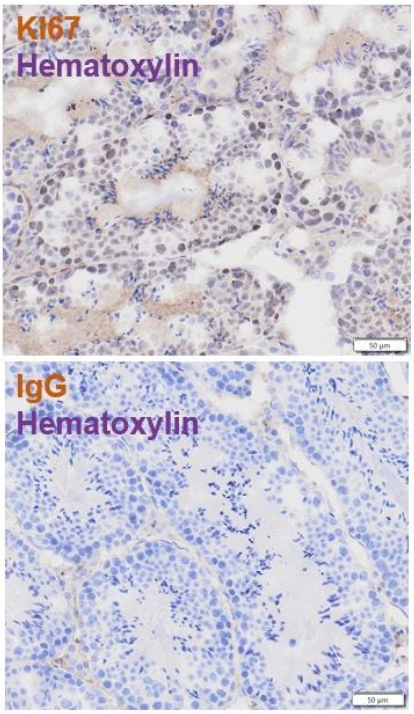

Figure 2. IHC analysis of Ki67 using anti- Ki67 antibody (BA0007) in mouse testis. Ki67 was detected in a paraffin-embedded section of mouse testis tissue. The tissue section was developed using HRP Conjugated Rabbit IgG with DAB as the chromogen.

Experiment Procedure

1. IF analysis of Ki67

(1) Reagent Preparation

Name	Batch Number/Source	Specifications
5%BSA	BAR0001	100g
10*PBS	BAR0002	500ml
TritonX-100	BAR0003	50ml
IGg	BAR0004/Rabbit	200μg
primary antibody	**BA0007/ Rabbit**	50μl
Secondary antibody	BAR0003 /Rabbit	0.5ml

Solution Preparation:

1x PBS: Dilute 200 ml of 10x PBS with ultrapure water to a total volume of 2 liters.

0.3% Triton X-100: Add 150 μl of Triton X-100 to 50 ml of PBS.

5% BSA: Dissolve 0.5 g of BSA in 10 ml of 1x PBS.

IgG Dilution Factor: For an antibody concentration of 1 mg/ml, the dilution factor is 1:200. For an IgG concentration of 1.67 mg/ml, the corresponding dilution factor is 1:334 (to maintain equal protein amounts).

(2) Experimental operation

1.Fixation: After removing the tissue (and rinsing thoroughly with PBS), immerse the tissue in FPA solution and store it at 4°C overnight. The next day, prepare a 3% sucrose solution (15 g sucrose + 50 ml PBS, mix well), add it to a centrifuge tube containing the tissue (just enough to cover the tissue, not too much), and store it at 4°C overnight. Replace the 3% sucrose solution daily until the tissue sinks to the bottom of the tube. Then, mix 3% sucrose with OCT compound at a 1:1 ratio, store it at 4°C overnight, and retrieve the tissue the next day for embedding.

2.Embedding: Take out the fixed tissue, use a blade to trim the desired section, and cut it to the required size. Add OCT compound to a PVC embedding mold (just enough to cover the bottom), place the tissue in the mold in the correct orientation for sectioning (cutting surface facing down), and fully cover the tissue with OCT compound. Freeze in a -20°C freezer for 5 minutes.

3.Cryosectioning: Remove the cryostat chuck and pre-cool the cryostat to -25°C. Demold the tissue block, attach it to the chuck using OCT compound, and freeze it in a -50°C freezer for 5 minutes. Use the cryostat to section the tissue (slice thickness 5 μm). Pick up the sections with adhesive slides (two spots per slide), label the slides, and place them on a staining rack. After sectioning, clean the cryostat (note: wipe the OCT compound off the chuck with paper).

4.Slide Selection: After cryosectioning, select slides with clear structures and good morphology (without folds, cracks, or incomplete sections). Place the slides in a 37°C oven for 10 minutes.

5.Gel Washing: Pour 1x PBS into a staining jar, and wash the slides with 1x PBS three times, for 8 minutes each. After washing, gently blot off excess liquid with paper.

6.Permeabilization: Place the slides in a humidified chamber, add 0.3% Triton X-100, and incubate for 10 minutes. Then, wash the slides in 1x PBS on a shaker three times, for 5 minutes each. Membrane proteins do not require permeabilization (note: ensure the shaker speed is not too fast, as it may cause tissue detachment). After washing, gently blot off excess liquid with paper.

7.Blocking: Use a hydrophobic pen to circle the sample (draw a circle that is neither too large nor too small, ensuring it doesn't touch the tissue). Place the slide in a humidified chamber, add 5% BSA, and incubate for 1 hour at room temperature. After incubation, gently blot off excess liquid with paper.

8.Primary Antibody: Place the slide in a humidified chamber, add the primary antibody diluted in 5% BSA, and incubate overnight at 4°C (Ki67 dilution ratio 1:100, i.e., 0.5 μl primary antibody + 50 μl 5% BSA; follow the antibody manual for reference). For control tissue, add IgG (IgG ratio: 1:167). Note: If the slide has two spots, select one as the control group. Use IgG matching the species of the primary antibody. The next day, remove the slides from the 4°C fridge and wash in 1x PBS on a shaker three times, for 8 minutes each. After washing, gently blot off excess liquid with paper.

9.Secondary Antibody: Place the slide in a humidified chamber, add the secondary antibody diluted in 5% BSA, and incubate for 1 hour at room temperature, protected from light (Ki67 dilution ratio 1:400, i.e., 1 μl secondary antibody + 400 μl 5% BSA; channel: 568 Cy3). After incubation, wash the slide in 1x PBS on a shaker three times, for 8 minutes each, protected from light. After washing, gently blot off excess liquid with paper. (Note: the secondary antibody must match the primary antibody. For example, if the primary is from Rat, select an anti-Rat secondary antibody and note the labeled channel on the secondary antibody.)

10.Counterstaining: Add fluorescence mounting medium containing DAPI to the slide, and counterstain for 10 minutes, protected from light (ensure the tissue is fully covered). Carefully check for air bubbles; if present, gently remove them with a pipette without touching the tissue.

11.Mounting: Cover the tissue with a coverslip, and dry the slide in a 37°C oven for 30 minutes. When placing the coverslip, gently lower one side first, then slowly lower the other side by hand. Do this at a moderate speed to avoid creating air bubbles.

2. IHC analysis of Ki67

(1) Reagent Preparation

Name	Batch Number/Source	Specifications
5%BSA	BAR0001	100g
10*PBS	BAR0002	500ml
IGg	BAR0004/ Rabbit	200μg
primary antibody	**BA0007/ Rabbit**	50μl
DAB staining solution	BAR0006	18ml
TritonX-100	BAR0003	50ml
Tris-EDTA Antigen Retrieval Solution	BAR0007	100ml

Solution Preparation:

5% BSA: Dissolve 0.5 g of BSA in 7 ml of PBS, then dilute to a final volume of 10 ml.

1x PBS: Dilute 200 ml of 10x PBS with ultrapure water to a total volume of 2 liters, then add 500 μl of Tween.

IgG Dilution Factor: For an antibody concentration of 1 mg/ml, the dilution factor is 1:200. For an IgG concentration of 1.67 mg/ml, the corresponding dilution factor is 1:334 (to maintain equal protein amounts).

(2) Experimental operation

1.Fixation: Add OCT compound to a PVC embedding mold, place the tissue in the mold in the correct orientation for sectioning (cutting surface facing down), and freeze in a -20°C freezer for 5 minutes.

2.Cryosectioning: Remove the cryostat chuck and pre-cool the cryostat to -25°C. Demold the tissue block, attach it to the chuck using OCT compound, and freeze it in a -50°C freezer for 5 minutes. Use the cryostat to section the tissue (slice thickness 10 μm). Pick up the sections with adhesive slides (three spots per slide), label the slides, and place them on a staining rack. After sectioning, clean the cryostat (note: wipe the OCT compound off the chuck with paper).

3.Slide Selection: After cryosectioning, select slides with clear structures and good morphology (without folds, cracks, or incomplete sections). Place the slides in a 37°C oven for 10 minutes.

4.Gel Washing: Pour 1x PBS into a staining jar, and wash the slides with 1x PBS three times, for 8 minutes each. After washing, gently blot off excess liquid with paper.

5.Antigen Retrieval: Use EDTA-TRIS/citric acid antigen retrieval solution. After bringing the solution to a boil, immerse the slides, reduce the heat to low, and incubate for 20 minutes. Allow the slides to cool naturally to room temperature, then wash with PBS for 5 minutes, twice.

6.Blocking Agent: Incubate the slides with hydrogen peroxide at room temperature for 20 minutes, then wash with PBS for 5 minutes, twice.

7.Blocking: Place the slides in a humidified chamber, add 5% BSA, and incubate for 1 hour at room temperature.

After incubation, gently blot off excess liquid with paper.

8.Primary Antibody: Place the slides in a humidified chamber, add the primary antibody diluted in 5% BSA, and incubate overnight at 4°C (ki67 dilution ratio 1:200, i.e., 0.1 μl primary antibody + 100 μl 5% BSA; follow the antibody manual for guidance). For control tissue, add IgG (IgG ratio: 1:334). Note: If the slide has three spots, select one as the control group. Use IgG matching the species of the primary antibody. The next day, remove the slides from the 4°C fridge and wash in 1x PBS on a shaker three times, for 8 minutes each. After washing, gently blot off excess liquid with paper.

9.Polymer: Place the slides in a humidified chamber, add enzyme-labeled anti-mouse/rabbit polymer, and incubate for 1 hour at room temperature. After incubation, wash the slides with 1x PBS on a shaker three times, for 5 minutes each, protected from light. After washing, gently blot off excess liquid with paper. (Note: the secondary antibody must match the primary antibody. For example, if the primary is from Rat, select an anti-Rat secondary antibody and ensure the correct channel is selected.)

10.DAB Staining: Add a sufficient amount of freshly prepared DAB chromogen solution (1:20 or 1:40). Adjust the staining time according to the desired intensity, then immerse the slides in ultrapure water for 5 minutes. (Time:30s)

11.Hematoxylin Counterstaining: Filter hematoxylin through a 0.45 mm filter before use. Adjust staining time based on desired intensity; if too dark, use a differentiation solution. Immerse the slides in ultrapure water for 3 minutes. (Time:10s)

12.Dehydration: Place the slides in 95% ethanol for 5 minutes, then transfer to absolute ethanol for 5 minutes, repeating the absolute ethanol step.

13.Mounting: Place the slides in xylene until no bubbles remain, then add an appropriate amount of neutral resin and cover with a coverslip. Air dry for 15 minutes in the fume hood, then transfer to an oven for 2 hours (perform all steps inside the fume hood).