Anti- Vimentin Antibody (Clone# SC60-05)

Catalog Number: *BA0011*

BIOACE

Bio'Ace BIOLOGICAL TECHNOLOGY
1013 Centre Road, Suite 403S, Wilmington, DE 19805, County of New Castle, Delaware, USA

Web: www.ibioace.com *Email:* bioace88@gmail.com

Basic Information

Product Name	Anti-Vimentin Antibody (Clone#SC60-05)
Gene Name	Vimentin
Source	Rabbit
Clonality	Monoclonal
Isotype	IgG
Species Reactivity	human, mouse, rat
Tested Application	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC, IHC-Fr
Contents	1ug/ul in 1×TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Immunogen	Synthetic peptide within C-terminal human Vimentin.
Concentration	1ug/ul
Purification	Protein A affinity purified
Observed MW	54 kDa

Recommended Dilution Ratios

Application	Dilution Ratios
WB	1: 20,000
IF-Tissue	1:1,000
IF-Cell	1:100-1:500
IHC-P	1:1,000-1:10,000
IHC-Fr	1:50-1:1,000
FC	1;1,000
IP	Use at an assay dependent concentration

Storage

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

Background Information

Vimentin (57 kDa) is the most ubiquituos intermediate filament protein and the first to be expressed during cell differentiation. All primitive cell types express vimentin but in most non-mesenchymal cells it is replaced by other intermediate filament proteins during differentiation. Vimentin is expressed in a wide variety of mesenchymal cell types: fibroblasts, endothelial cells etc., and in a number of other cell types derived from mesoderm, e.g., mesothelium and ovarian granulosa cells. Vimentin is present in many different neoplasms but is particulary

expressed in those originated from mesenchymal cells. Sarcomas e.g., fibrosarcoma, malignt fibrous histiocytoma, angiosarcoma, and leio- and rhabdomyosarcoma, as well as lymphomas, malignant melanoma and schwannoma, are virtually always vimentin positive. Mesoderm derived carcinomas like renal cell carcinoma, adrenal cortical carcinoma and adenocarcinomas from endometrium and ovary usually express vimentin. Also thyroid carcinomas are vimentin positive. Any low differentiated or sarcomatoid carcinoma may express some vimentin. Vimentin is frequently included in the so-called primary panel (together with CD45, cytokeratin, and S-100 protein): Intense staining reaction for vimentin without coexpression of other intermediate filament proteins is strongly suggestive of a mesenchymal tumour or a malignant melanoma. However, in biopsies representing only a sarcomatoid part of renal cell carcinoma a.o. a strong positivity for vimentin without cytokeratin expression may be seen. Tumours like lymphomas and seminomas have the same intermediate filament profile, but the vimentin expression is usually weaker.

Selected Validation Data

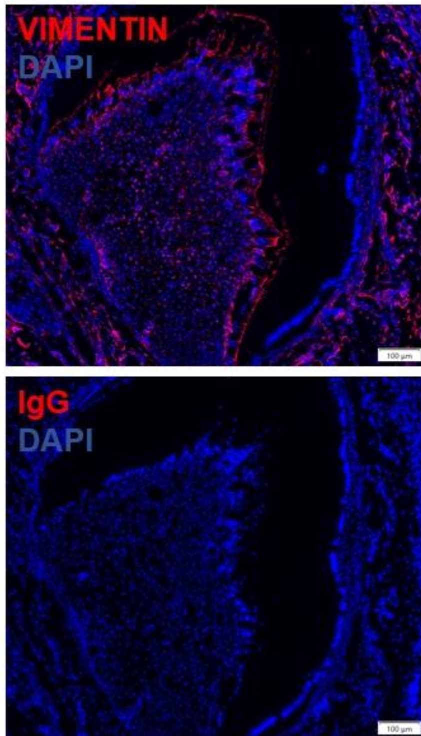

Figure 1. IF analysis of Vimentin using anti-Vimentin antibody (BA0011) in mouse teeth. Vimentin was detected in a paraffin-embedded section of mouse teeth tissue. 568 cy3 conjugated Anti- Rabbit IgG Secondary Antibody (Cyan) was used as secondary antibody. The section was counterstained with DAPI (Blue).

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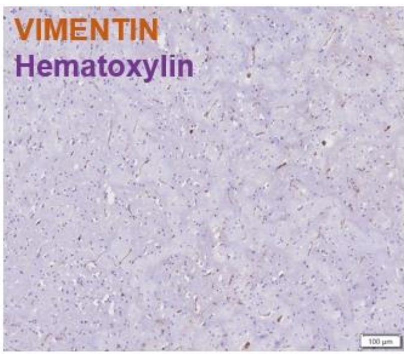
<td>

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<td>Figure 2. IHC analysis of Vimentin using anti-Vimentin antibody (BA0011) in mouse brain. Vimentin was detected in a paraffin-embedded section of mouse brain tissue. The tissue section was developed using HRP Conjugated Rabbit IgG with DAB as the chromogen.</td>
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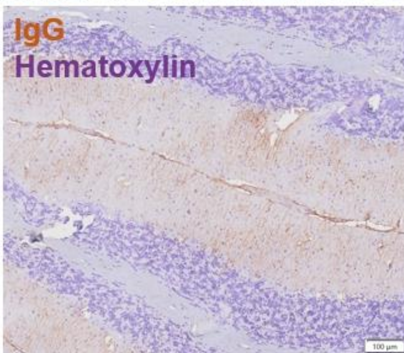
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<td>Figure 3. IHC analysis of Vimentin using anti- Vimentin antibody (BA0011) in mouse brain. Vimentin was detected in a paraffin-embedded section of mouse brain tissue. The tissue section was developed using HRP Conjugated Rabbit IgG with DAB as the chromogen.</td>
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Experiment Procedure

1. IF analysis of Vimentin

(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	**BA0011/ 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 washing it clean with PBS), immerse the tissue in FPA solution and place it in a 4°C refrigerator overnight. On the second day, prepare a 3% sucrose solution (15g sucrose + 50ml PBS, mix well) and add it to the centrifuge tube containing the tissue (just enough to cover the tissue, not too much). Store the tube in the 4°C refrigerator overnight. Change the 3% sucrose solution daily until the tissue sinks to the bottom of the tube. Then, mix 3% sucrose with OCT in a 1:1 ratio, store it in a 4°C refrigerator overnight, and take it out the next day for embedding.

2.Embedding: After fixation, use a scalpel to cut the desired tissue sections and size. Add OCT to a PVC embedding mold (just enough to cover the base, not too much). Place the tissue in the mold according to the cutting direction (cutting surface down), then squeeze OCT to fully cover the tissue. Freeze in a -20°C freezer for 5 minutes.

3.Cryosectioning: Remove the button from the cryostat, pre-cool the cryostat to -25°C, and demold the tissue block. Attach the tissue block to the button using OCT, then place the button in a -50°C freezer for 5 minutes. Use the cryostat to cut sections (section thickness: 10 μm). Use adhesive slides to pick up the sections (two spots per slide). Label the slides and place them in a staining rack. After completing the sections, clean the cryostat (note: wipe the OCT off the button with tissue paper).

4.Slide Selection: After freezing the sections, select slides with clear structure and good morphology (no overlapping, tearing, or incomplete sections). Place them in a 37°C oven for 10 minutes.

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

6.Permeabilization: Place the slides in a humidified chamber and add 0.3% Triton, incubating for 10 minutes. Then, wash the slides in 1x PBS on a shaker three times, 5 minutes each (note: no permeabilization is required for membrane proteins). After washing, gently blot the excess liquid with tissue paper.

7.Blocking: Use a hydrophobic pen to circle the sample on the slide (aim to draw a circle, not too large or too small, and avoid damaging the tissue). Place the slides in a humidified chamber and add 5% BSA, incubating at room temperature for 1 hour. After incubation, gently blot the excess liquid with tissue paper.

8.Primary Antibody: Place the slides in a humidified chamber and add the primary antibody diluted in 5% BSA, incubating overnight at 4°C (for Vimentin: brain tissue 1:1000, 0.1 μl primary antibody + 100 μl 5% BSA; tooth tissue 1:400, 0.5 μl primary antibody + 200 μl 5% BSA, based on the antibody datasheet). For control tissue, add IgG (IgG dilution: brain 0.1:167, tooth 0.5:334). Note: If the slide has two spots, select one for the control group, and use IgG corresponding to the source of the primary antibody. The next day, take the slides out of the 4°C refrigerator and wash in 1x PBS on a shaker three times, 8 minutes each. After washing, gently blot the excess liquid with tissue paper.

9.Secondary Antibody: Place the slides in a humidified chamber and add the secondary antibody diluted in 5% BSA, incubating for 1 hour at room temperature in the dark (for Vimentin: 1:400, i.e., 1 μl secondary antibody + 400 μl 5% BSA, channel: 568 cy3). After incubation, wash the slides in 1x PBS on a shaker three times, 8 minutes each, protected from light. After washing, gently blot the excess liquid with tissue paper (note: the secondary antibody must match the primary antibody; for example, if the primary antibody is Rat-derived, select an anti-Rat secondary antibody and ensure the correct channel is used).

10.Counterstaining: Add fluorescent mounting medium containing DAPI and incubate in the dark for 10 minutes (just enough to fully cover the tissue). Carefully check for bubbles and use a pipette to remove them if present, being careful not to touch the tissue.

11.Mounting: Place a coverslip on top of the slide and dry the slides in a 37°C oven for 0.5 hours. When placing the coverslip, gently lower one side first and then carefully lower the other side to avoid generating bubbles.

2. IHC analysis of Vimentin

(1) Reagent Preparation

Name	Batch Number/Source	Specifications
5%BSA	BAR0001	100g
10*PBS	BAR0002	500ml
IGg	BAR0004/ Rabbit	200μg
primary antibody	**BA0011/ 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 into the mold following the cutting direction (cutting surface down), and freeze in a -20°C freezer for 5 minutes.

2.Cryosectioning: Remove the button from the cryostat, pre-cool the cryostat to -25°C, demold the tissue block, and adhere it to the button with OCT compound. Freeze the button in a -50°C freezer for 5 minutes. Cut sections using the cryostat (section thickness: 10 μm) and use adhesive slides to pick up the sections (three spots per slide). Label the slides and place them in a staining rack. After sectioning, clean the cryostat (note: wipe the OCT compound off the button with tissue).

3.Slide Selection: After freezing the sections, select slides with clear structure and good morphology (no overlapping, tears, or incomplete sections), and place them in a 37°C oven for 10 minutes.

4.Gel Washing: Pour 1x PBS into a staining jar, wash the slides in 1x PBS three times, each for 8 minutes, then gently blot the excess liquid with tissue.

5.Antigen Retrieval: Boil EDTA-TRIS/citrate antigen retrieval buffer, place the slides in the solution, and reduce heat to low for 20 minutes. Allow the slides to cool to room temperature, then wash with PBS for 5 minutes, two times.

6.Blocking: Incubate the slides in hydrogen peroxide blocking solution at room temperature for 20 minutes, followed by two PBS washes, 5 minutes each.

7.Blocking: Place the slides in a humidified chamber and add 5% BSA, incubating for 1 hour at room temperature. After incubation, gently blot the excess liquid with tissue.

8.Primary Antibody: Place the slides in a humidified chamber and add the primary antibody diluted in 5% BSA. Incubate overnight at 4°C (Vimetin dilution ratio: 1:1000, i.e., 0.1 μl primary antibody + 100 μl 5% BSA, dilution based on the antibody datasheet). For control tissue, add IgG (IgG dilution: 1:1667). If the slide has three spots, use one as a control group and choose the IgG based on the source of the primary antibody. The next day, wash the slides three times with 1x PBS on a shaker, 8 minutes per wash. After washing, gently blot the excess liquid with tissue.

9.Polymer: Place the slides in a humidified chamber, add HRP-labeled polymer anti-mouse/rabbit, and incubate for 1 hour at room temperature. Wash the slides three times with 1x PBS on a shaker in the dark, 5 minutes each. After washing, gently blot the excess liquid with tissue (note: the secondary antibody should match the primary antibody; if the primary antibody is from Rat, use an anti-Rat secondary antibody and confirm the correct channel).

10.DAB Staining: Add an appropriate amount of freshly prepared DAB staining solution (dilution 1:20 or 1:40). Adjust the staining time based on the desired staining effect. After staining, wash in Up water for 5 minutes. (Time:20s)

11.Hematoxylin Counterstaining: Filter the hematoxylin solution with a 0.45 mm filter before use. Adjust the staining time according to the staining effect. If the staining is too dark, use differentiation solution. Wash in Up water for 3 minutes. (Time:10s)

12.Dehydration: Place the slides in 95% ethanol for 5 minutes, transfer to absolute ethanol for 5 minutes, and repeat with fresh absolute ethanol for another 5 minutes.

13.Mounting: Place the slides in xylene until no bubbles remain. Add an appropriate amount of neutral resin and cover with a coverslip. Air dry in a fume hood for 15 minutes, then transfer to an oven for 2 hours (the entire process should be performed in a fume hood).

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