Anti-CD34 Antibody (Clone# SI16-01)

Catalog Number: *BA0002*

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Basic Information

Product Name	Anti-CD34 Antibody (Clone# SI16-01)
Gene Name	CD34
Source	Rabbit
Clonality	Monoclonal
Isotype	IgG
Species Reactivity	human, mouse, rat
Tested Application	WB, IF-Cell, IF-Tissue, IHC-P, IP, mIHC, FC
Contents	1ug/ul in 1×TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Immunogen	Synthetic peptide within Human CD34
Concentration	1ug/ul
Purification	Protein A affinity purified
Observed MW	Predicted band size: 41 kDa

Recommended Dilution Ratios

Application	Dilution Ratios
WB	1:2,000
IF-Tissue	1:500-1:1,000
IF-Cell	1:100
IHC-P	1:400-1:10,000
FC	1:1,000
IP	1-2μg /sample
mIHC	1:1,000-1:2,000

Storage

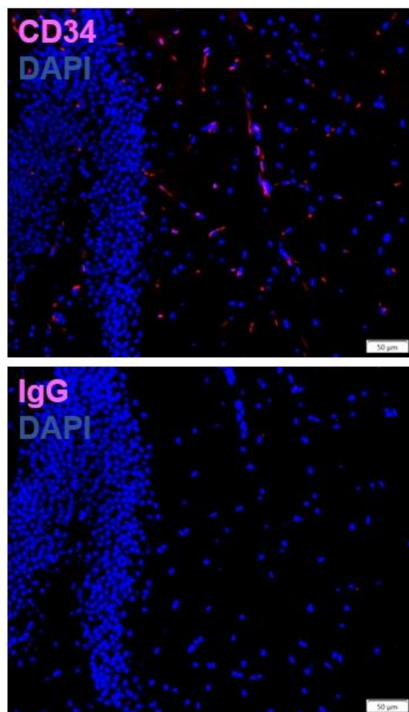
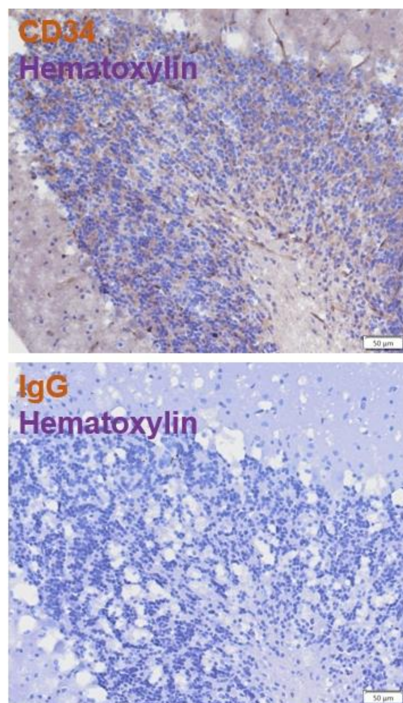
Store at +4°C after thawing. Aliquot store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

Background Information

CD34 is a monomeric cell surface antigen with a molecular mass of approximately 110 KD.CD34 is expressed in humans in hematopoietic stem cells, vascular endothelium, and blasts from 30% of patients with acute myeloid and lymphocytic leukemia. The human CD34 gene spans 26 kb and has 8 exons, a structure quite similar to that of the murine gene. By Southern blot analysis of DNA from a panel of human x mouse somatic cell hybrids using a CD34 cDNA probe demonstrate that the gene for CD34 is located on human chromosome 1 in the 1q12----qter

region. CD34 plays an important role in the formation of progenitor cells during both embryonic and adult hematopoiesis.

Selected Validation Data

	Figure 1. IF analysis of CD34 using anti-CD34 antibody (BA0002) in mouse hippocampus. CD34 was detected in a paraffin-embedded section of mouse hippocampus 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 CD34 using anti-CD34 antibody (BA0002) in mouse hippocampus. CD34 was detected in a paraffin-embedded section of mouse hippocampus tissue. The tissue section was developed using HRP Conjugated Rabbit IgG with DAB as the chromogen.

Experiment Procedure

1. IF analysis of CD34

(1) Reagent Preparation

Name	Batch Number/Source	Specifications
5%BSA	BAR0001	100g
10*PBS	BAR0002	500ml
IGg	BAR0004/ Rabbit	200μg
primary antibody	**BA0002/ Rabbit**	50μl
Secondary antibodies	BAR0005/ Rabbit	0.5ml

Solution Preparation:

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

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 it 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, and store it at 4°C overnight. The next day, retrieve the tissue for embedding.

2.Embedding: Take out the fixed tissue, use a blade to cut the desired section, and trim it to the desired 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), then 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 10 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.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.

7.Primary Antibody: Place the slide in a humidified chamber, add the primary antibody diluted in 5% BSA, and incubate overnight at 4°C (CD34 dilution ratio 1:1000, i.e., 0.1 μl primary antibody + 100 μl 5% BSA; follow the antibody manual for guidance). For control tissue, add IgG (IgG ratio: 0.1:167). 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.

8.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 (CD34 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 a rat, select an anti-rat secondary antibody, and note the labeled channel on the secondary antibody).

9.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.

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

2. IHC analysis of CD34

(1) Reagent Preparation

Name	Batch Number/Source	Specifications
5%BSA	BAR0001	100g
10*PBS	BAR0002	500ml
IGg	BAR0004/ Rabbit	200μg
primary antibody	**BA0002/ 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 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 and reduce the heat 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 (CD34 dilution ratio 1:1000, i.e., 0.1 μl primary antibody + 100 μl 5% BSA; follow the antibody manual for guidance). For control tissue, add IgG (IgG ratio: 1:1667). 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 the 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, protecting them 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 desired intensity, then immerse in ultrapure water for 5 minutes.

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

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 in the fume hood for 15 minutes, then transfer to an oven for 2 hours (perform all steps inside the fume hood).