Mouse Reactive M1 vs M2 Macrophage Antibody Sampler Kit

HAK21096

Contains Product	Quantity	Applications	Species reactivity	MW(kDa)
F4/80 [HA721520]	20µl	WB,IHC-P,IF-Tissue,IHC-Fr,mIHC	M,R	102 kDa
CD68 [HA722285]	20µl	WB,IF-Cell,IHC-P,IHC-Fr,IF-Tissue	M,R	35 kDa
CD86 [ET1606-50]	20µl	WB,IF-Cell,IP	H,M,R	38 kDa
CD11c [HA722830]	20µl	WB,IHC-P,IHC-Fr,IF-Tissue	M	129 kDa
Mannose Receptor(CD206) [HA722892]	20µl	WB,IHC-P,IF-Tissue,IHC-Fr,IF-Cell,IP,mIHC	M,R	165 kDa
Liver Arginase [HA723143]	20µl	WB,IHC-P,IF-Tissue	H,M,R	35 kDa
Iba1 [ET1705-78]	20µl	WB,IHC-P,IHC-Fr,IF-Tissue,IF-Cell,FC,IP,mIHC	H,M,R	17 kDa
HRP-Goat Anti-Rabbit IgG (H+L) [HA1001]	100µl	WB,ELISA,IHC-P	Rab	

Description: The Mouse Reactive M1 vs M2 Macrophage IHC Antibody Sampler Kit provides an economical means of characterizing the extent of M1 and M2 macrophages in formalin-fixed, paraffin-embedded tissue samples.

Storage Buffer: PBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Background Macrophages are myeloid cells of the innate immune system that are found in all human tissues in the body and exhibit anatomical and functional diversity. These heterogenous cells are derived from monocyte precursors in the blood that infiltrate into the tissues and differentiate in the presence of cytokines and growth factors. A spectrum of different macrophage phenotypes, or polarizations, have been described based on their secretory profiles, gene expression, and functions. Macrophages have great plasticity and can switch from one phenotype to another under different conditions. At the opposite extremes of this spectrum are so called M1, or classically activated phenotype, and M2 or alternatively activated phenotype. M1 macrophages are generally inflammatory and anti-tumor, while M2 macrophages, commonly referred to as tumor-associated macrophages (TAMs), are generally anti-inflammatory and pro-tumor. Relative contents of M1 and M2 macrophages in the tumor microenvironment may have prognostic values. Modulating macrophage polarization is actively pursued as a therapeutic intervention for many different diseases. In mice, F4/80, CD68, and Iba1/AIF-1 are considered general markers for macrophages. CD86, CD11c, and others have been used as markers for M1 macrophages, while CD206, Arginase-1, and others have been used as markers for M2 macrophages.

Database links: UniProt ID: Q61549, Q5Y4N8, P31996, 287435, P42081, P42082, 56822, Q9QXH4, P20702, Q61830, 291327, P05089, Q61176, P07824, P55008, O70200, P55009

Images

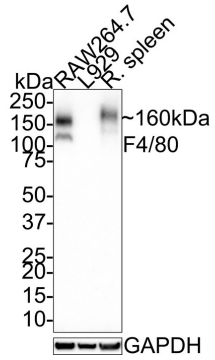

Fig1: Western blot analysis of F4/80 on different lysates with Rabbit anti-F4/80 antibody (HA721520) at 1/1,000 dilution.

Lane 1: RAW264.7 cell lysate (no heat) (20 µg/Lane)
Lane 2: L929 cell lysate (no heat) (negative) (20 µg/Lane)
Lane 3: Rat spleen tissue lysate (70℃ heat) (40 µg/Lane)

Notice: no heat means the lysate is not boiled.

Predicted band size: 102 kDa
Observed band size: 160 kDa

Exposure time: 2 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721520) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

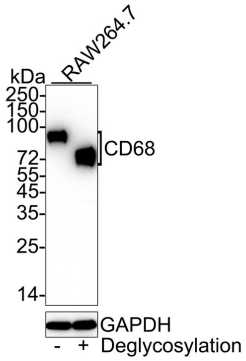

Fig2: Western blot analysis of CD68 on different lysates with Rabbit anti-CD68 antibody (HA722285) at 1/1,000 dilution.

Lane 1: RAW264.7 cell lysate
Lane 2: RAW264.7 cell lysate treated with deglycosylation

Lysates/proteins at 20 µg/Lane.

Predicted band size: 37 kDa
Observed band size: 90/70 kDa

Exposure time: 25 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722285) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

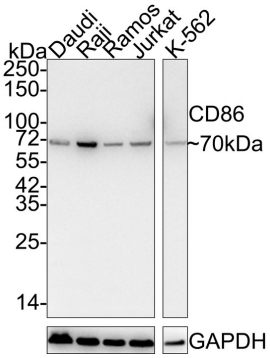

Fig3: Western blot analysis of CD86 on different lysates with Rabbit anti-CD86 antibody (ET1606-50) at 1/2,000 dilution.

Lane 1: Daudi cell lysate, 15 µg/Lane
Lane 2: Raji cell lysate, 15 µg/Lane
Lane 3: Ramos cell lysate, 15 µg/Lane
Lane 4: Jurkat cell lysate, 15 µg/Lane
Lane 5: K-562 cell lysate, 15 µg/Lane

Predicted band size: 38 kDa
Observed band size: 70 kDa

Exposure time: 2 minutes 37 seconds;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1606-50) at 1/2,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

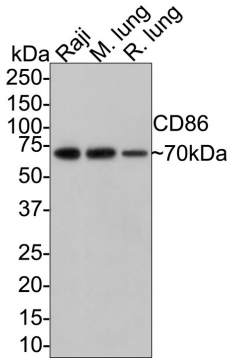

Fig4: Western blot analysis of CD86 on different lysates with Rabbit anti-CD86 antibody (ET1606-50) at 1/1,000 dilution.

Lane 1: Raji cell lysate (10 µg/Lane)
Lane 2: Mouse lung tissue lysate (20 µg/Lane)
Lane 3: Rat lung tissue lysate (20 µg/Lane)

Predicted band size: 38 kDa
Observed band size: 70 kDa

Exposure time: 1 minute;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1606-50) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

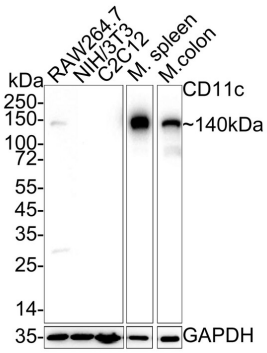

Fig5: Western blot analysis of CD11c on different lysates with Rabbit anti-CD11c antibody (HA722830) at 1/1,000 dilution.

Lane 1: RAW264.7 cell lysate (20 µg/Lane)
Lane 2:NIH/3T3 cell lysate (negative) (20 µg/Lane)
Lane 3: C2C12 cell lysate (negative) (20 µg/Lane)
Lane 4: Mouse spleen tissue lysate (30 µg/Lane)
Lane 5: Mouse colon tissue lysate (30 µg/Lane)

Predicted band size: 129 kDa
Observed band size: 140 kDa

Exposure time: Lane1-3: 3 minutes ; Lane 4-5: 25 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722830) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

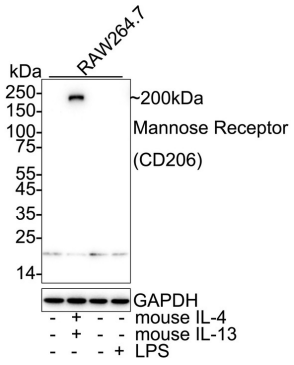

Fig6: Western blot analysis of Mannose Receptor(CD206) on different lysates with Rabbit anti-Mannose Receptor(CD206) antibody (HA722892) at 1/2,000 dilution.

Lane 1: RAW264.7 cell lysate
Lane 2: RAW264.7 treated with 20ng/mL mIL-4 and 10ng/mL mIL-13 for 24 hours cell lysate (Macrophage M2 polarization, positive)
Lane 3: RAW264.7 cell lysate
Lane 4: RAW264.7 treated with 0.1µg/mL LPS for 6 hours cell lysate (Macrophage M1 polarization, negative)

Lysates/proteins at 20 µg/Lane.

Predicted band size: 165 kDa
Observed band size: 200 kDa

Exposure time: 59 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722892) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

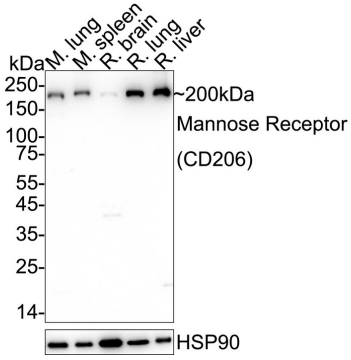

Fig7: Western blot analysis of Mannose Receptor(CD206) on different lysates with Rabbit anti-Mannose Receptor(CD206) antibody (HA722892) at 1/1,000 dilution.

Lane 1: Mouse lung tissue lysate (20 µg/Lane)
Lane 2: Mouse spleen tissue lysate (20 µg/Lane)
Lane 3: Rat brain tissue lysate (20 µg/Lane)
Lane 4: Rat lung tissue lysate (20 µg/Lane)
Lane 5: Rat liver tissue lysate (20 µg/Lane)

Predicted band size: 165 kDa
Observed band size: 200 kDa

Exposure time: 42 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722892) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

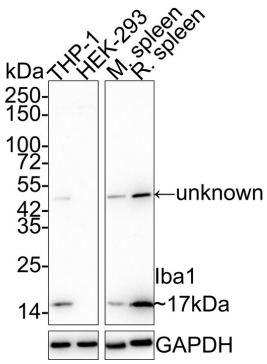

Fig8: Western blot analysis of Iba1 on different lysates with Rabbit anti-Iba1 antibody (ET1705-78) at 1/5,000 dilution.

Lane 1: THP-1 cell lysate, 20 µg/Lane
Lane 2: HEK-293 cell lysate (negative), 20 µg/Lane
Lane 3: Mouse spleen tissue lysate, 20 µg/Lane
Lane 4: Rat spleen tissue lysate, 20 µg/Lane

Predicted band size: 17 kDa
Observed band size: 17 kDa

Exposure time: 3 minutes;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1705-78) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

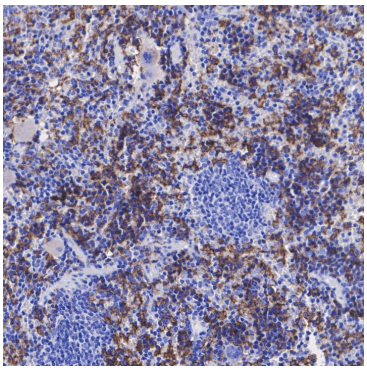

Fig9: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-F4/80 antibody (HA721520) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721520) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

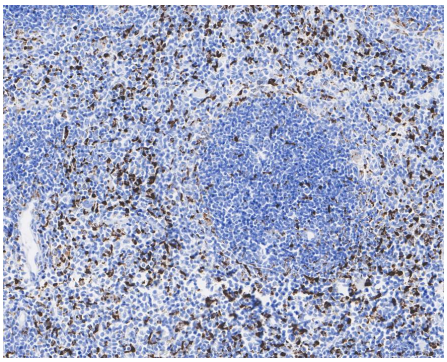

Fig10: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-CD68 antibody (HA722285) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722285) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

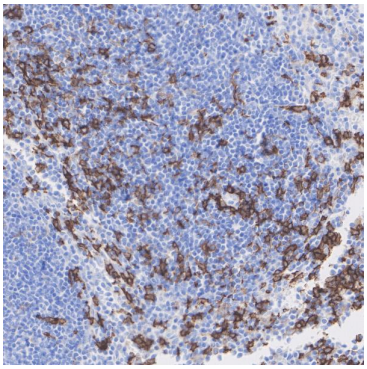

Fig11: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-CD11c antibody (HA722830) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722830) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

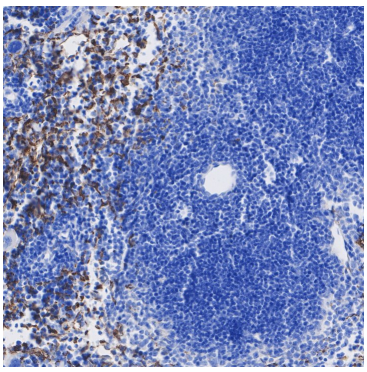

Fig12: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-Mannose Receptor(CD206) antibody (HA722892) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA722892) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

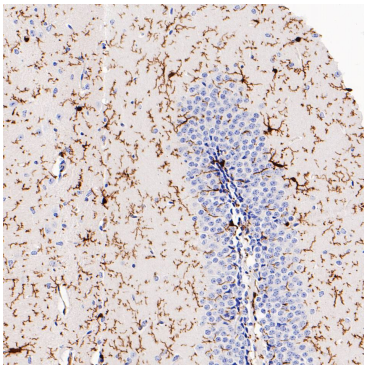

Fig13: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-Iba1 antibody (ET1705-78) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1705-78) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

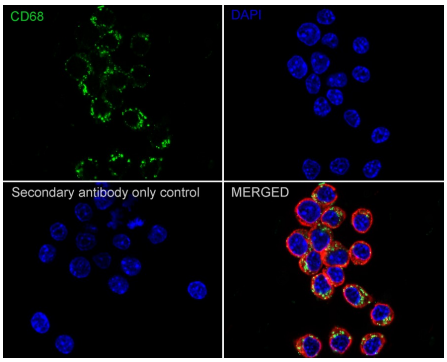

Fig14: Immunocytochemistry analysis of RAW264.7 cells labeling CD68 with Rabbit anti-CD68 antibody (HA722285) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-CD68 antibody (HA722285) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

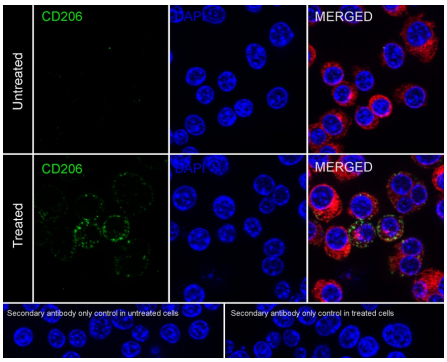

Fig15: Immunocytochemistry analysis of RAW264.7 cells treated with 20ng/mL mIL-4 and 10ng/mL mIL-13 for 24 hours (Macrophage M2 polarization, positive) labeling Mannose Receptor(CD206) with Rabbit anti-Mannose Receptor(CD206) antibody (HA722892) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Mannose Receptor(CD206) antibody (HA722892) at 1/50 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

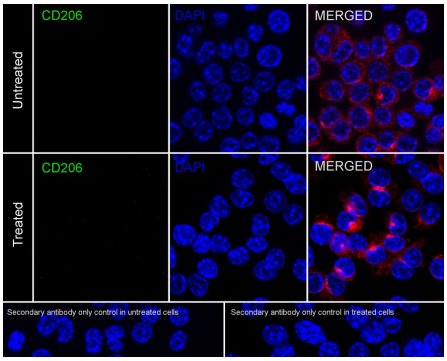

Fig16: Immunocytochemistry analysis of RAW264.7 cells treated with 0.1μg/mL LPS for 6 hours (Macrophage M1 polarization, negative) labeling Mannose Receptor(CD206) with Rabbit anti-Mannose Receptor(CD206) antibody (HA722892) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Mannose Receptor(CD206) antibody (HA722892) at 1/50 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

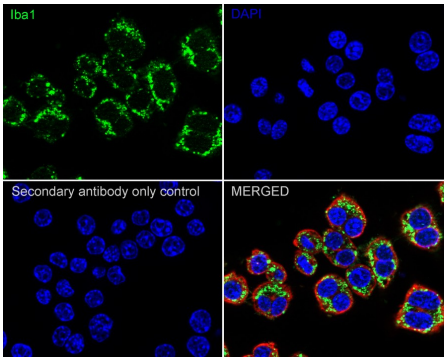

Fig17: Immunocytochemistry analysis of RAW264.7 cells labeling Iba1 with Rabbit anti-Iba1 antibody (ET1705-78) at 1/250 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Iba1 antibody (ET1705-78) at 1/250 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI. Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

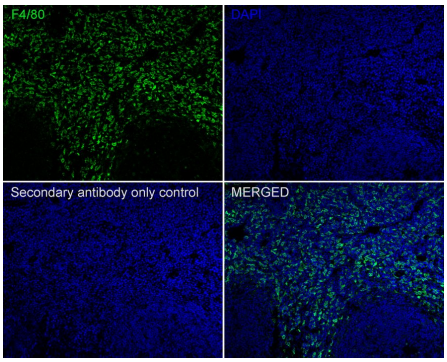

Fig18: Immunofluorescence analysis of frozen mouse spleen tissue with Rabbit anti-F4/80 antibody (HA721520) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721520, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

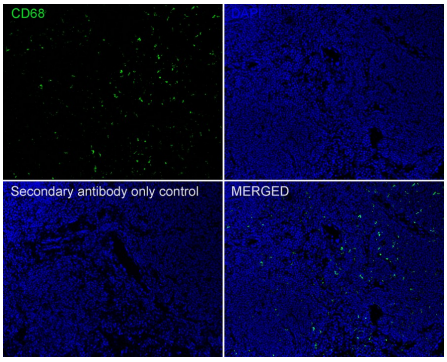

Fig19: Immunofluorescence analysis of frozen mouse lymph nodes tissue with Rabbit anti-CD68 antibody (HA722285) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA722285, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

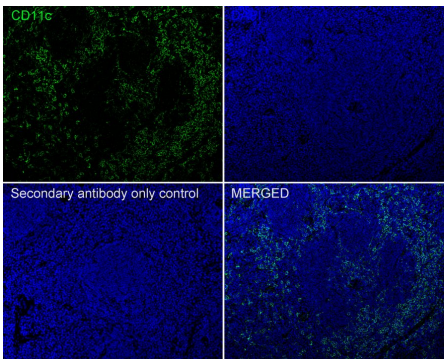

Fig20: Immunofluorescence analysis of frozen mouse spleen tissue with Rabbit anti-CD11c antibody (HA722830) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA722830, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

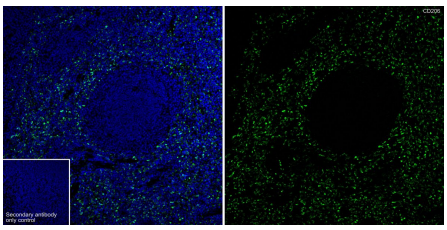

Fig21: Immunofluorescence analysis of frozen mouse spleen tissue with Rabbit anti-Mannose Receptor(CD206) antibody (HA722892) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA722892, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

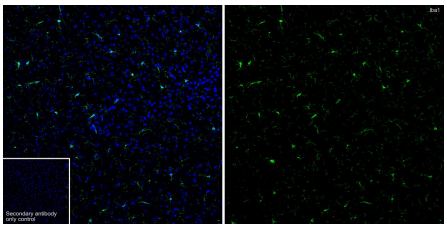

Fig22: Immunofluorescence analysis of frozen mouse brain tissue with Rabbit anti-Iba1 antibody (ET1705-78) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1705-78, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".