Anti-PBR Antibody [SA90-03]

ET1601-19

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 19 kDa
Clone number:	SA90-03

Description: Translocator protein (TSPO) is an 18 kDa protein mainly found on the outer mitochondrial membrane. It was first described as peripheral benzodiazepine receptor (PBR), a secondary binding site for diazepam, but subsequent research has found the receptor to be expressed throughout the body and brain. In humans, the translocator protein is encoded by the TSPO gene. It belongs to a family of tryptophan-rich sensory proteins. Regarding intramitochondrial cholesterol transport, TSPO has been proposed to interact with StAR (steroidogenic acute regulatory protein) to transport cholesterol into mitochondria, though evidence is mixed. In animals, TSPO (PBR) is a mitochondrial protein usually located in the outer mitochondrial membrane and characterised by its ability to bind a variety of benzodiazepine-like drugs, as well as to dicarboxylic tetrapyrrole intermediates of the haem biosynthetic pathway. TSPO has many proposed functions depending on the tissue. The most studied of these include roles in the immune response, steroid synthesis and apoptosis.

Immunogen: Synthetic peptide within Human PBR aa 120-169 / 169.

Positive control: HCT 116 cell lysate, THP-1 cell lysate, 293T cell lysate, SK-OV-3 cell lysate, NIH/3T3 cell lysate, RAW264.7 cell lysate, PC-12 cell lysates, THP-1, human colon tissue, human kidney tissue, mouse colon tissue, mouse kidney tissue, human colon carcinoma tissue, human prostate carcinoma tissue.

Subcellular location: Mitochondrion membrane

Database links: SwissProt: P30536 Human | P50637 Mouse | P16257 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:1,000
IF-Tissue	1:50-1:200
IHC-P	1:400-1:1,000
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

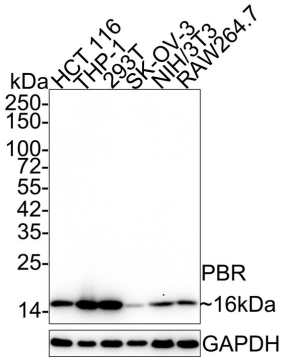

Fig1: Western blot analysis of PBR on different lysates with Rabbit anti-PBR antibody (ET1601-19) at 1/2,000 dilution.

Lane 1: HCT 116 cell lysate
Lane 2: THP-1 cell lysate
Lane 3: 293T cell lysate
Lane 4: SK-OV-3 cell lysate
Lane 5: NIH/3T3 cell lysate
Lane 6: RAW264.7 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 19 kDa
Observed band size: 16 kDa

Exposure time: 1 minute 4 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 (ET1601-19) 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.

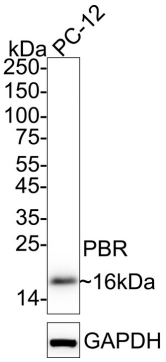

Fig2: Western blot analysis of PBR on PC-12 cell lysates with Rabbit anti-PBR antibody (ET1601-19) at 1/2,000 dilution.

Lysates/proteins at 15 µg/Lane.

Predicted band size: 19 kDa
Observed band size: 16 kDa

Exposure time: 4 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 (ET1601-19) 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.

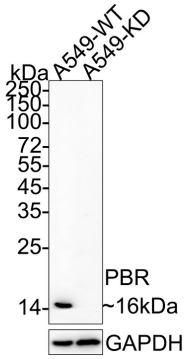

Fig3: Western blot analysis of PBR on different lysates with Rabbit anti-PBR antibody (ET1601-19) at 1/2,000 dilution.

Lane 1: A549-si NT cell lysate
Lane 2: A549-si PBR cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 19 kDa
Observed band size: 16 kDa

Exposure time: 1 minute 51 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 (ET1601-19) 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 1/50,000 dilution was used for 1 hour at room temperature.

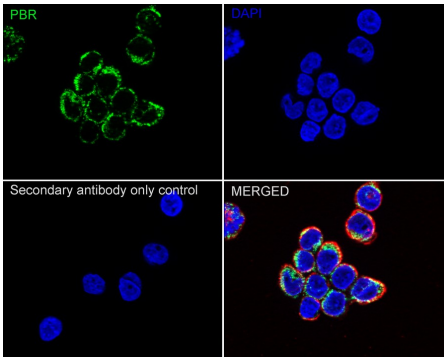

Fig4: Immunocytochemistry analysis of THP-1 cells labeling PBR with Rabbit anti-PBR antibody (ET1601-19) at 1/1,000 dilution.

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-PBR antibody (ET1601-19) at 1/1,000 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.

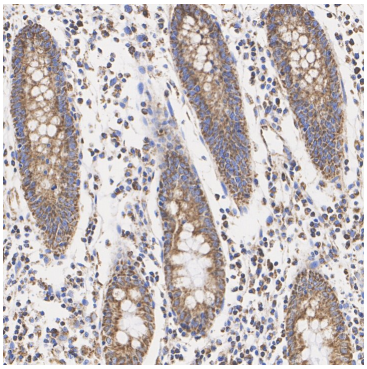

Fig5: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-PBR antibody (ET1601-19) at 1/1,000 dilution.

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 (ET1601-19) 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.

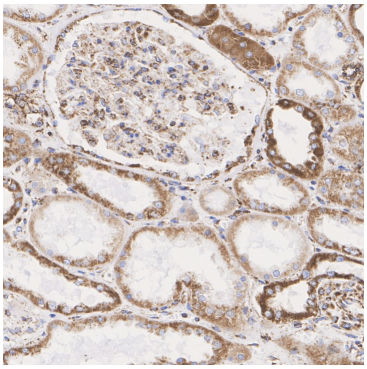

Fig6: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-PBR antibody (ET1601-19) at 1/1,000 dilution.

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 (ET1601-19) 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.

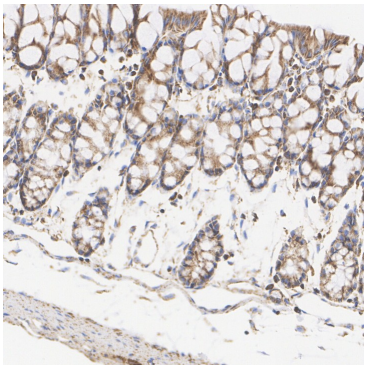

Fig7: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-PBR antibody (ET1601-19) at 1/1,000 dilution.

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 (ET1601-19) 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.

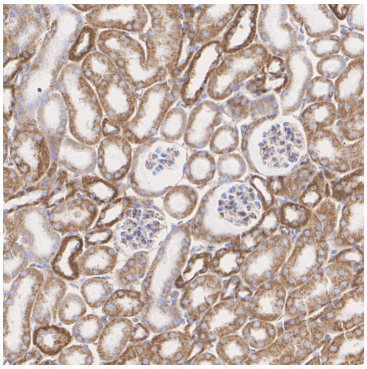

Fig8: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-PBR antibody (ET1601-19) at 1/1,000 dilution.

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 (ET1601-19) 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.

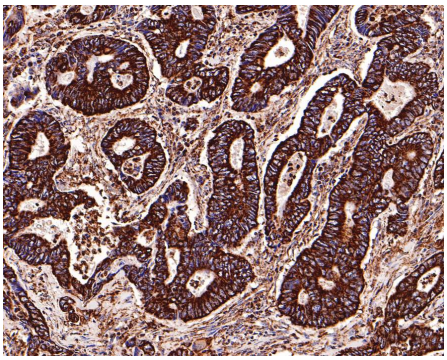

Fig9: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-PBR antibody (ET1601-19) at 1/400 dilution.

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 (ET1601-19) at 1/400 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.

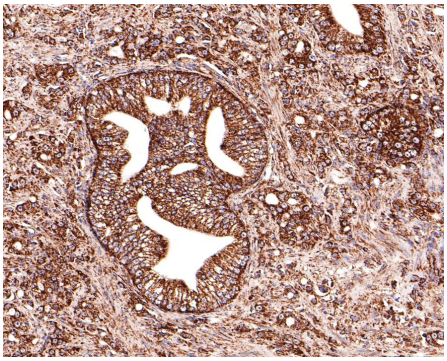

Fig10: Immunohistochemical analysis of paraffin-embedded human prostate carcinoma tissue with Rabbit anti-PBR antibody (ET1601-19) at 1/400 dilution.

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 (ET1601-19) at 1/400 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.

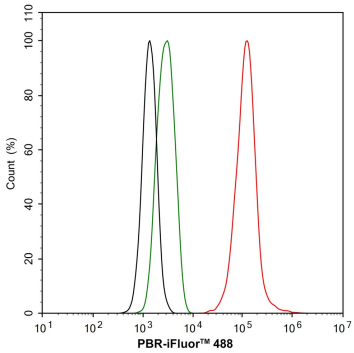

Fig11: Flow cytometric analysis of THP-1 cells labeling PBR.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1601-19, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

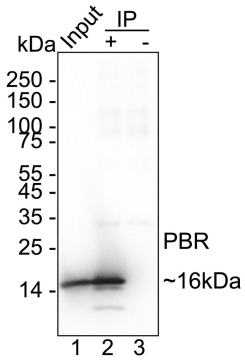

Fig12: PBR was immunoprecipitated from 0.2 mg THP-1 cell lysate with ET1601-19 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using ET1601-19 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: THP-1 cell lysate (input)
Lane 2: ET1601-19 IP in THP-1 cell lysate
Lane 3: Rabbit IgG instead of ET1601-19 in THP-1 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 1 seconds; ECL: K1801

Background References

1. Tóth M et al. Acute neuroinflammation in a clinically relevant focal cortical ischemic stroke model in rat: longitudinal positron emission tomography and immunofluorescent tracking. Brain Struct Funct N/A:N/A (2015).
2. Banati RB et al. Positron emission tomography and functional characterization of a complete PBR/TSPO knockout. Nat Commun 5:5452 (2014).