abcam

Product datasheet

Anti-Piccolo antibody ab20664

★★★★★ 4 Abreviews 12 References 画像数 4

製品の概要

製品名	Anti-Piccolo antibody
製品の詳細	Rabbit polyclonal to Piccolo
由来種	Rabbit
特異性	Due to its large size, Piccolo requires special gel-electrophoresis and Western blotting protocols for visualization by immunoblotting. Excellent results can be obtained, for example, with the 4-12% TRIS-glycine gradient gels of Anamed. For success in WB with Rabbit polyclonal to Piccolo - Synaptic Marker (ab20664), do not denature WB sample lysate.
アプリケーション	**適用あり:** IHC (PFA fixed), WB, IHC-P
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
特記事項	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
バッファー	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
精製度	Immunogen affinity purified

ポリ/モノ	ポリクローナル
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab20664の使用に適用されます

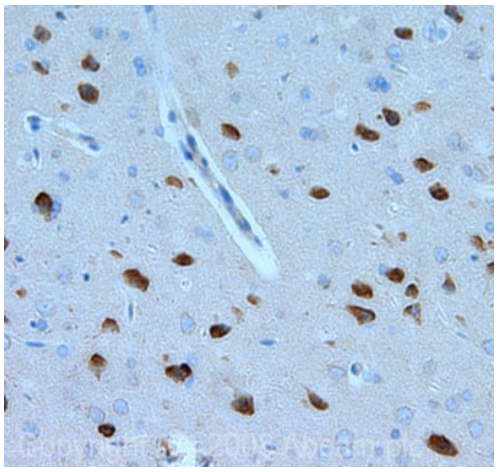
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
IHC (PFA fixed)		1/300 - 1/1000.
WB	★★★★★ (1)	Use a concentration of 0.2 - 0.5 µg/ml. Detects a band of approximately 460 kDa (predicted molecular weight: 520 kDa). Abcam recommends using milk as the blocking agent.
IHC-P	★★★★☆ (1)	Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

ターゲット情報

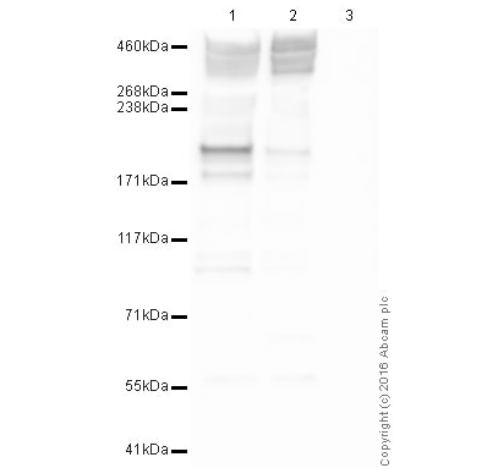
機能	May act as a scaffolding protein involved in the organization of synaptic active zones and in synaptic vesicle trafficking.
配列類似性	Contains 2 C2 domains. Contains 1 PDZ (DHR) domain.
ドメイン	C2 domain 1 is involved in binding calcium and phospholipids. Calcium binds with low affinity but with high specificity and induces a large conformational change.
細胞内局在	Cell junction > synapse. Concentrated at the presynaptic side of synaptic junctions.

画像



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Piccolo antibody (ab20664)

IHC image of Piccolo staining in rat brain FFPE section, performed on a Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab20664, 5µg/ml, for 8 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.



Western blot - Anti-Piccolo antibody (ab20664)

All lanes : Anti-Piccolo antibody (ab20664) at 1 µg/ml

Lane 1 : Brain (Rat) Tissue Lysate
Lane 2 : Cerebellum (Rat) Tissue Lysate
Lane 3 : Kidney (Rat) Tissue Lysate (Negative control)

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/50000 dilution

Developed using the ECL technique.

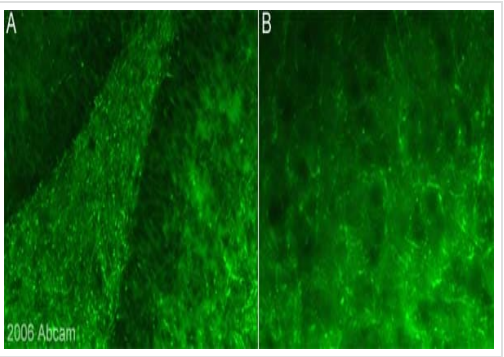
Performed under reducing conditions.

Predicted band size: 520 kDa
Observed band size: 460 kDa
Additional bands at: 200 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 4 minutes

The banding pattern shown in the image above is consistent with the literature which describes multiple bands >420 kDa as a result of proteolysis (PMID : 10707984).

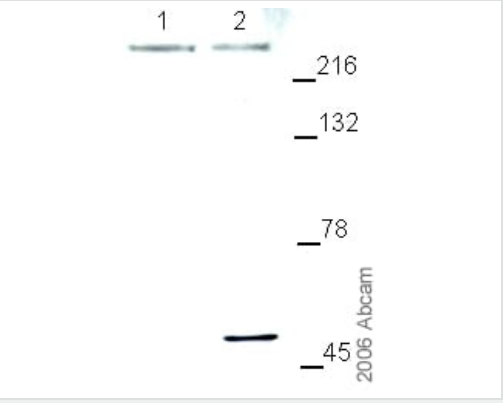
This blot was produced using a 3-8% Tris Acetate gel under the TA buffer system. The gel was run at 150V for 60 minutes using lysates heated to 85°C before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% Milk before being incubated with ab20664 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution **ab133406**.



Immunohistochemistry (PFA fixed) - Anti-Piccolo antibody (ab20664)

This image is courtesy of Sophie Pezet, King's College London, United Kingdom

Immuofluorescent staining for Piccolo ab20664 in [A] rat brain hippocampus (X20 objective) and [B] rat brain cortex (X40 objective). Tissue preparation: rat brain tissue was perfusion fixed (4% PFA) followed by post fix and cryoprotection in 20% sucrose before freezing in OCT. 30µm coronal sections were cut on a cryostat for free floating IHC. Primary antibody ab20664 was used at 1/100 (5µg/ml) incubated overnight at room temperature in PBST (triton 0.3%). Secondary antibody used: anti-rabbit Alexa fluor 488 (1/1000) incubated for 2 hours at room temperature.



Western blot - Anti-Piccolo antibody (ab20664)

This image is courtesy of Randal Moldrich, CNRS UMR7637, ESPCI, France

All lanes : Anti-Piccolo antibody (ab20664) at 0.2 µg/ml

Lane 1 : Rat brain lysate at 40 µg
Lane 2 : Mouse brain lysate at 50 µg

Secondary
All lanes : Anti-rabbit HRP at 1/20000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 520 kDa
Observed band size: 520 kDa
Additional bands at: 60 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 10 minutes

Primary Antibody : anti-Piccolo (ab20664) for 1h in 5% Whole Milk

Powder (WMP); Secondary Antibody : Anti-rabbit HRP (1/20000) for 1h in 5% WMP; Migration medium: Laemmeli + glycerol + 5% B-mercaptoethanol; Transfer : Tris/Glycine, 20% ethanol

NB: Gels higher than 8% acrylamide were tried without success; 8% or lower is recommended. Denaturing of samples at 70C or 95C was not successful. Reducing conditions were used and 20% ethanol was employed for the nitrocellulose membrane transfer. All steps performed at RT.

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