Human/Mouse/Rat Phospho-ERK1(T202/Y204)/ERK2 (T185/Y187) Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG

Catalog Number: AF1018

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse and rat Phospho-ERK1/ERK2 when dually phosphorylated at T202/Y204 and T185/Y187, respectively.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	Phosphopeptide containing ERK1 T202/Y204 sites
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

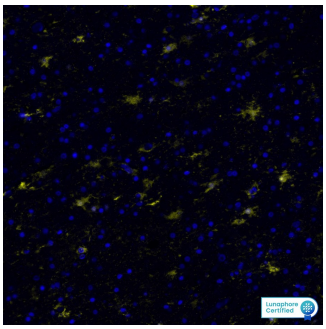
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.1 µg/mL	See Below
Flow Cytometry	2.5 µg/10^6 cells	See Below
Multiplex Immunofluorescence	25 µg/mL	Immersion fixed paraffin-embedded sections of human brain cortex and mouse cerebellum
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	5 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

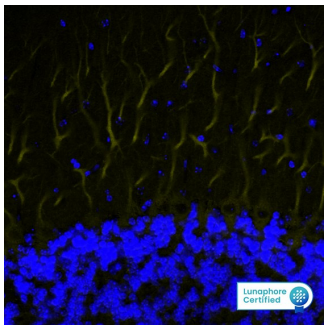
DATA

Multiplex Immunofluorescence



Detection of Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) in Human Brain Cortex via seqIF™ staining on COMET™
Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) was detected in immersion fixed paraffin-embedded sections of human Cortex using Rabbit Anti-Human Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) , Polyclonal Antibody (Catalog #AF1018) at 25ug/mL at 37 ° Celsius for 8 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9; Epredia Catalog # TA-999-DHBH). Tissue was stained using the Alexa Fluor™ Plus 647 Goat anti-Rabbit IgG Secondary Antibody at 1:200 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR647RB) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the cytoplasm and nucleus of the neuron. Protocol available in COMET™ Panel Builder.

Multiplex Immunofluorescence



Detection of Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) in Mouse Cerebellum via seqIF™ staining on COMET™
Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) was detected in immersion fixed paraffin-embedded sections of mouse Cerebellum using Rabbit Anti-Mouse Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187), Polyclonal Antibody (Catalog #AF1018) at 25ug/mL at 37° Celsius for 4 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9; Epredia Catalog # TA-999-DHBH). Tissue was stained using the Alexa Fluor™ Plus 555 Goat anti-Rabbit IgG Secondary Antibody at 1:100 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR555RB) and counterstained with DAPI (blue; Lunaphore Catalog # DR100). Specific staining was localized to the cytoplasm of the neuronal processes. Protocol available in COMET™ Panel Builder.

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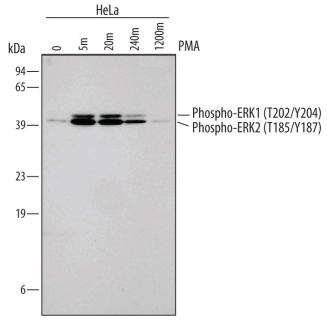
Human/Mouse/Rat Phospho-ERK1(T202/Y204)/ERK2 (T185/Y187) Antibody

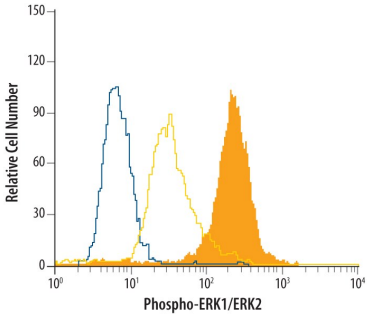
Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF1018

Western Blot



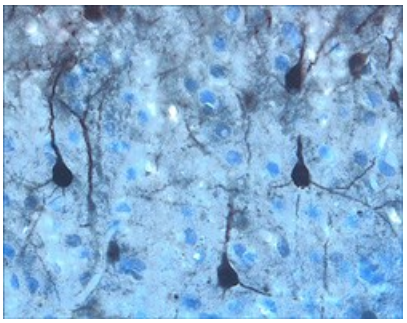
Detection of Human Phospho-ERK1 (T202/Y204) and ERK2 (T185/Y187) by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line untreated (-) or treated (+) with 200 nM PMA for for the indicated times. PVDF membrane was probed with 0.1 µg/mL of Rabbit Anti-Human/Mouse/Rat Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1018), followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # Catalog # HAF008). Specific bands were detected for Phospho-ERK1 (T202/Y204) and ERK2 (T185/Y187) at approximately 42 and 44 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



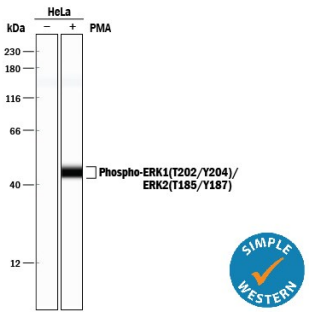
Detection of Phospho-ERK1/ERK2 in Jurkat Human Cell Line by Flow Cytometry. Jurkat human acute T cell leukemia cell line were untreated (yellow line open histogram) or treated with 200 ng/mL PMA for 15 minutes (filled histogram) and stained with Rabbit Anti-Human/Mouse/Rat Phospho-ERK1/ERK2 (ERK1 T202/Y204, ERK2 T185/Y187) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1018) or control antibody (Catalog # Catalog # AB-105-C, blue line open histogram), followed by Fluorescein-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # Catalog # F0112). To facilitate intracellular staining, cells were fixed with PFA and permeabilized with ice-cold methanol.

Immunohistochemistry



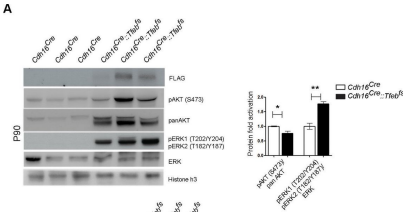
ERK1/ERK2 in Rat Brain. ERK1/ERK2 was detected in perfusion fixed frozen sections of rat brain (cortex) using 15 µg/mL Rabbit Anti-Human/Mouse/Rat Phospho-ERK1/ERK2 (ERK1 T202/Y204, ERK2 T185/Y187) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1018) overnight at 4 °C. Tissue was stained with the Anti-Rabbit HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # Catalog # CTS005) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Frozen Tissue Sections.

Simple Western



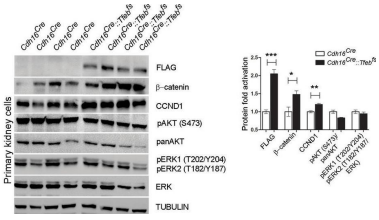
Detection of Human Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) by Simple Western™. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line untreated (-) or treated (+) with PMA, loaded at 0.2 mg/mL. A specific band was detected for ERK1 (T202/Y204)/ERK2 (T185/Y187) at approximately 44 kDa (as indicated) using 5 µg/mL of Rabbit Anti-Human/Mouse/Rat Phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1018). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Western Blot



Detection of Mouse ERK1/ERK2 by Western Blot Biochemical analysis of ErbB signaling.Immunoblot analysis performed on P90 kidneys from Cdh16Cre::Tfebfs mice (A) and P90 Cdh16CreErt2::Tfebfs animals induced with tamoxifen at P14 (B) and at P30 (C), respectively. Each replicate is a different biological sample. ErbB was analyzed by quantifying phosphoAKT (Ser473) to total AKT, and phosphoERK1 (T202/Y204)/ERK2(T185/Y187) to total ERK; graphs are the densitometry quantifications of Western blot bands normalized to wild-type line and are shown as an average (± SEM) (*p<0.05, **p<0.01, ***p<0.001, two-sided Student's t test).DOI:https://dx.doi.org/10.7554/eLife.17047.011 Image collected and cropped by CiteAb from the following publication

Western Blot



Detection of Mouse ERK1/ERK2 by Western Blot Activation of ErbB and WNT signaling pathways in kidneys from Cdh16Cre::Tfebfs mice.Transcriptional and biochemical analyses were performed on Cdh16Cre and Cdh16Cre::Tfebfs mice. (A,B) Tables show the relative increase of genes related to the ErbB (A) and WNT (B) pathways in the microarray analyses performed on kidneys from P0 Cdh16Cre::Tfebfs mice. Graphs show real-time PCR validations performed on kidneys from Cdh16Cre::Tfebfs mice at different stages (P0, P12, P30). Data are shown as the average (± SEM) of at least three Cdh16Cre::Tfebfs mice normalized versus wild-type mice. (C,D) Immunoblot analyses performed on (C) P30 kidney tissues and (D) primary kidney cells isolated from

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Cdh16Cre::Tfebfs mice to evaluate ErbB and WNT activation status. Each replicate is a distinct biological sample. ErbB signaling was assessed by looking at phosphoAKT (Ser473) to total AKT ratio, and phosphoERK1 (T202/Y204)/ERK2(T185/Y187) to total ERK ratio; WNT signaling was assessed by quantifying β-catenin and CCND1 (Cyclin D1) protein levels. Graphs represent the densitometry quantification of Western blot bands. Values are normalized to actin when not specified and are shown as an average (± SEM) (*p<0.05, **p<0.01, ***p<0.001, two-sided, Student's t test).DOI:https://dx.doi.org/10.7554/eLife.17047.00710.7554/eLife.17047.008Figure 3—source data 1.Complete list of 294 genes (represented by 361 probesets) significantly induced (FDR≤0.05) in the KSP_P0 microarray dataset (GSE62977).The genes are ranked by decreasing signed ratio (KSP_P0/CTL).DOI:https://dx.doi.org/10.7554/eLife.17047.00810.7554/eLife.17047.009Figure 3—source data 2.Complete list of 628 genes (represented by 729 probesets) significantly induced (FDR≤0.05) in the KSP_P14 microarray dataset (GSE63376).The genes are ranked by decreasing signed ratio (KSP_P14/CTL).DOI:https://dx.doi.org/10.7554/eLife.17047.009Complete list of 294 genes (represented by 361 probesets) significantly induced (FDR≤0.05) in the KSP_P0 microarray dataset (GSE62977).The genes are ranked by decreasing signed ratio (KSP_P0/CTL).DOI:https://dx.doi.org/10.7554/eLife.17047.008Complete list of 628 genes (represented by 729 probesets) significantly induced (FDR≤0.05) in the KSP_P14 microarray dataset (GSE63376).The genes are ranked by decreasing signed ratio (KSP_P14/CTL).DOI:https://dx.doi.org/10.7554/eLife.17047.009ErbB and WNT transcriptional profiles in Cdh16CreErt2::Tfebfs mice.Transcriptional analyses performed on Cdh16CreErt2::Tfebfs mice. (A,B) mRNA levels of previously validated genes belonging to the WNT (left graphs) and ErbB (right graphs) signaling pathways assessed in P90 Cdh16CreErt2::Tfebfs mice induced at (A) P14 and at (B) P30 with tamoxifen respectively. Data are shown as the average (± SEM) of at least Cdh16CreErt2::Tfebfs mice and values are normalized to the wild-type line. (*p<0.05, **p<0.01, ***p<0.001, two-sided Student's t test).DOI:https://dx.doi.org/10.7554/eLife.17047.010Biochemical analysis of ErbB signaling.Immunoblot analysis performed on P90 kidneys from Cdh16Cre::Tfebfs mice (A) and P90 Cdh16CreErt2::Tfebfs animals induced with tamoxifen at

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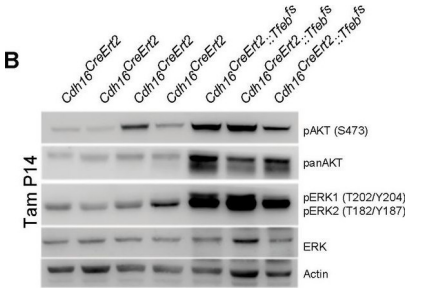
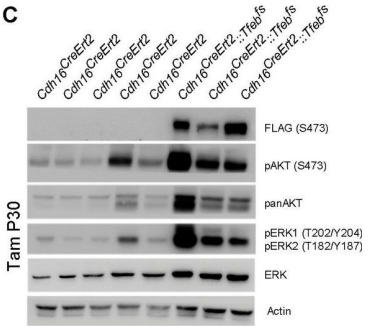
P14 (B) and at P30 (C), respectively. Each replicate is a different biological sample. ErbB was analyzed by quantifying phosphoAKT (Ser473) to total AKT, and phosphoERK1 (T202/Y204)/ERK2(T185/Y187) to total ERK; graphs are the densitometry quantifications of Western blot bands normalized to wild-type line and are shown as an average (± SEM) (*p<0.05, **p<0.01, ***p<0.001, two-sided Student's t test).DOI:https://dx.doi.org/10.7554/eLife.17047.011 Image collected and cropped by CiteAb from the following publication (https://elifesciences.org/articles/17047), licensed under a CC-BY license. Not internally tested by R&D Systems.

Western Blot



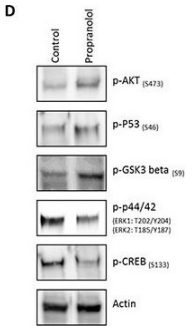
Detection of Mouse ERK1/ERK2 by Western Blot
Biochemical analysis of ErbB signaling.Immunoblot analysis performed on P90 kidneys from Cdh16Cre::Tfebfs mice (A) and P90 Cdh16CreErt2::Tfebfs animals induced with tamoxifen at P14 (B) and at P30 (C), respectively. Each replicate is a different biological sample. ErbB was analyzed by quantifying phosphoAKT (Ser473) to total AKT, and phosphoERK1 (T202/Y204)/ERK2(T185/Y187) to total ERK; graphs are the densitometry quantifications of Western blot bands normalized to wild-type line and are shown as an average (± SEM) (*p<0.05, **p<0.01, ***p<0.001, two-sided Student's t test).DOI:https://dx.doi.org/10.7554/eLife.17047.011 Image collected and cropped by CiteAb from the following publication (https://elifesciences.org/articles/17047), licensed under a CC-BY license. Not internally tested by R&D Systems.

Western Blot



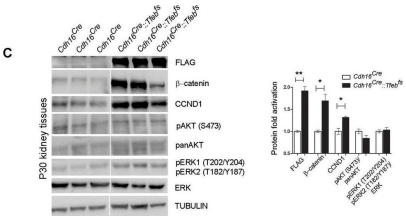
Detection of Mouse ERK1/ERK2 by Western Blot
Biochemical analysis of ErbB signaling.Immunoblot analysis performed on P90 kidneys from Cdh16Cre::Tfebfs mice (A) and P90 Cdh16CreErt2::Tfebfs animals induced with tamoxifen at P14 (B) and at P30 (C), respectively. Each replicate is a different biological sample. ErbB was analyzed by quantifying phosphoAKT (Ser473) to total AKT, and phosphoERK1 (T202/Y204)/ERK2(T185/Y187) to total ERK; graphs are the densitometry quantifications of Western blot bands normalized to wild-type line and are shown as an average (± SEM) (*p<0.05, **p<0.01, ***p<0.001, two-sided Student's t test).DOI:https://dx.doi.org/10.7554/eLife.17047.011 Image collected and cropped by CiteAb from the following publication (https://elifesciences.org/articles/17047), licensed under a CC-BY license. Not internally tested by R&D Systems.

Western Blot



Detection of Human ERK1/ERK2 by Western Blot
β-blockers inhibit the proliferation of breast cancer cell lines(A) A dose curve of propranolol was administered to a panel of breast cancer cell lines and normal primary mammary epithelial cells (HMECS) and viability was assessed. (B) Immunofluorescent detection of Ki-67 protein expression in control or propranolol-treated (18 μM) SK-BR-3 breast cancer cells after 24 hours. Hoechst 33342 was used as a nuclear counterstain. (C) Heatmap depicting phospho-MAPK antibody array results testing the status of 24 kinases in SK-BR-3 cells subjected to 24 hours control or propranolol (18 μM). (red = upregulated; green =

Western Blot



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Activation of ErbB and WNT signaling pathways in kidneys from Cdh16Cre::Tfebfs mice.Transcriptional and biochemical analyses were performed on Cdh16Cre and Cdh16Cre::Tfebfs mice. (A,B) Tables show the relative increase of genes related to the ErbB (A) and WNT (B) pathways in the microarray analyses performed on kidneys from P0 Cdh16Cre::Tfebfs mice. Graphs show real-time PCR validations performed on kidneys from Cdh16Cre::Tfebfs mice at different stages (P0, P12, P30). Data are shown as the average (± SEM) of at least three Cdh16Cre::Tfebfs mice normalized versus wild-type mice.

downregulated; black = no detectable expression). (D) Western blot confirmation of the antibody array results in SK-BR-3 cells subjected to 24 hours control or propranolol (18 µM). (D) Western blot analysis of cell lysates from SK-BR-3 cells subjected to 24 hours control or propranolol (18 µM), confirming the phosphorylation events identified in the antibody array. Image collected and cropped by CiteAb from the following publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.14119), licensed under a CC-BY license. Not internally tested by R&D Systems.

(C,D) Immunoblot analyses performed on (C) P30 kidney tissues and (D) primary kidney cells isolated from Cdh16Cre::Tfebfs mice to evaluate ErbB and WNT activation status. Each replicate is a distinct biological sample. ErbB signaling was assessed by looking at phosphoAKT (Ser473) to total AKT ratio, and phosphoERK1 (T202/Y204)/ERK2(T185/Y187) to total ERK ratio; WNT signaling was assessed by quantifying β-catenin and CCND1 (Cyclin D1) protein levels. Graphs represent the densitometry quantification of Western blot bands. Values are normalized to actin when not specified and are shown as an average (± SEM) (*p<0.05, **p<0.01, ***p<0.001, two-sided, Student's t test).DOI:https://dx.doi.org/10.7554/eLife.17047.00710.7554/eLife.17047.008Figure 3—source data 1.Complete list of 294 genes (represented by 361 probesets) significantly induced (FDR≤0.05) in the KSP_P0 microarray dataset (GSE62977).The genes are ranked by decreasing signed ratio (KSP_P0/CTL).DOI:https://dx.doi.org/10.7554/eLife.17047.00810.7554/eLife.17047.009Figure 3—source data 2.Complete list of 628 genes (represented by 729 probesets) significantly induced (FDR≤0.05) in the KSP_P14 microarray dataset (GSE63376).The genes are ranked by decreasing signed ratio (KSP_P14/CTL).DOI:https://dx.doi.org/10.7554/eLife.17047.009Complete list of 294 genes (represented by 361 probesets) significantly induced (FDR≤0.05) in the KSP_P0 microarray dataset (GSE62977).The genes are ranked by decreasing signed ratio (KSP_P0/CTL).DOI:https://dx.doi.org/10.7554/eLife.17047.008Complete list of 628 genes (represented by 729 probesets) significantly induced (FDR≤0.05) in the KSP_P14 microarray dataset (GSE63376).The genes are ranked by decreasing signed ratio (KSP_P14/CTL).DOI:https://dx.doi.org/10.7554/eLife.17047.009ErbB and WNT transcriptional profiles in Cdh16CreErt2::Tfebfs mice.Transcriptional analyses performed on Cdh16CreErt2::Tfebfs mice. (A,B) mRNA levels of previously validated genes belonging to the WNT (left graphs) and ErbB (right graphs) signaling pathways assessed in P90 Cdh16CreErt2::Tfebfs mice induced at (A) P14 and at (B) P30 with tamoxifen respectively. Data are shown as the average (± SEM) of at least Cdh16CreErt2::Tfebfs mice and values are normalized to the wild-type line. (*p<0.05, **p<0.01, ***p<0.001, two-sided Student's t test).DOI:https://dx.doi.org/10.7554/eLife.17047.010Biochemical analysis of ErbB signaling.Immunoblot analysis

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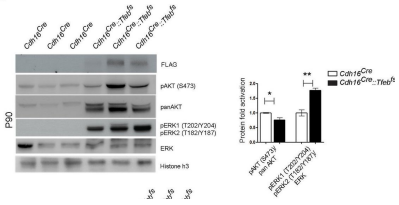
performed on P90 kidneys from Cdh16Cre::Tfebfs mice (A) and P90 Cdh16CreErt2::Tfebfs animals induced with tamoxifen at P14 (B) and at P30 (C), respectively. Each replicate is a different biological sample. ErbB was analyzed by quantifying phosphoAKT (Ser473) to total AKT, and phosphoERK1 (T202/Y204)/ERK2(T185/Y187) to total ERK; graphs are the densitometry quantifications of Western blot bands normalized to wild-type line and are shown as an average (± SEM) (*p<0.05, **p<0.01, ***p<0.001, two-sided Student's t test).DOI:https://dx.doi.org/10.7554/eLife.17047.011 Image collected and cropped by CiteAb from the following publication (https://elifesciences.org/articles/17047), licensed under a CC-BY license. Not internally tested by R&D Systems.

Western Blot

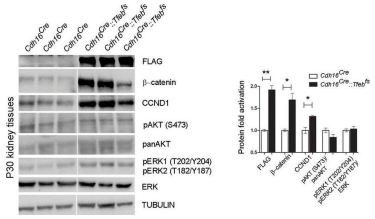
A



Detection of Mouse Human/Mouse/Rat Phospho-ERK1(T202/Y204)/ERK2 (T185/Y187) Antibody by Western Blot Biochemical analysis of ErbB signaling.Immunoblot analysis performed on P90 kidneys from Cdh16Cre::Tfebfs mice (A) and P90 Cdh16CreErt2::Tfebfs animals induced with tamoxifen at P14 (B) and at P30 (C), respectively. Each replicate is a different biological sample. ErbB was analyzed by quantifying phosphoAKT (Ser473) to total AKT, and phosphoERK1 (T202/Y204)/ERK2(T185/Y187) to total ERK; graphs are the densitometry quantifications of Western blot bands normalized to wild-type line and are shown as an average (± SEM) (*p<0.05, **p<0.01, ***p<0.001, two-sided Student's t test).DOI:http://dx.doi.org/10.7554/eLife.17047.011 Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27668431), licensed under a CC-BY license. Not internally tested by R&D Systems.

Western Blot

C



Detection of Mouse Human/Mouse/Rat Phospho-ERK1(T202/Y204)/ERK2 (T185/Y187) Antibody by Western Blot Activation of ErbB and WNT signaling pathways in kidneys from Cdh16Cre::Tfebfs mice.Transcriptional and biochemical analyses were performed on Cdh16Cre and Cdh16Cre::Tfebfs mice. (C,D) Immunoblot analyses performed on (C) P30 kidney tissues and (D) primary kidney cells isolated from Cdh16Cre::Tfebfs mice to evaluate ErbB and WNT activation status. Each replicate is a distinct biological sample. ErbB signaling was assessed by looking at phosphoAKT (Ser473) to total AKT ratio, and phosphoERK1 (T202/Y204)/ERK2(T185/Y187) to total ERK ratio; WNT signaling was assessed by quantifying β-catenin and CCND1 (Cyclin D1) protein levels. Graphs represent the densitometry quantification of Western blot bands. Values are normalized to actin when not specified and are shown as an average (± SEM) (*p<0.05, **p<0.01, ***p<0.001, two-sided, Student's t test). Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/27668431), licensed under a CC-BY license. Not internally tested by R&D Systems.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

ERK1 is a protein Serine/Threonine kinase that is a member of the extracellular signal-regulated kinases (ERKs) which are activated in response to numerous growth factors and cytokines (1). Activation of ERK1 requires both tyrosine and threonine phosphorylation that is mediated by MEK. ERK1 is ubiquitously distributed in tissues with the highest expression in heart, brain, and spinal cord. Activated ERK1 translocates into the nucleus where it phosphorylates various transcription factors.

References:

1. Roskoski Jr., R. (2012) Pharmacol Res. **66**:105.

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