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Datasheet for 600-401-421S

PPAR alpha Antibody

Overview

Description:	Anti-PPAR alpha (N-terminal specific) (RABBIT) Antibody - 600-401-421S
Item No.:	600-401-421S
Size:	25 µL
Applications:	ELISA, IF, IHC, WB, ChIP
Reactivity:	Human, Mouse
Host Species:	Rabbit

Product Details

Background:	Since their discovery in the early 1990's, the peroxisome proliferator activated receptors (PPARs) have attracted significant attention. This is primarily because PPARs serve as receptors for two very important classes of drugs: the hypolipidemic fibrates and the insulin sensitizing thiazolidinediones. Peroxisome proliferators are non-genotoxic carcinogens that are purported to exert their effect on cells through their interaction with members of the nuclear hormone receptor family termed PPARs. Nuclear hormone receptors are ligand-dependent intracellular proteins that stimulate transcription of specific genes by binding to specific DNA sequences following activation by the appropriate ligand. Upon binding fatty acids or hypolipidemic drugs, PPARs form heterodimers with retinoid X receptors (RXRs) and these heterodimers regulate the expression of target genes. There are 3 known subtypes of PPARs: PPAR-alpha, PPAR-delta and PPAR-gamma. Mostly target genes are involved in the catabolism of fatty acids. Conversely, PPAR-gamma is activated by peroxisome proliferators such as prostaglandins, leukotrienes and Anti diabetic thiazolidinediones and affects the expression of genes involved in the storage of the fatty acids. PPAR-gamma may also be involved in adipocyte differentiation. It has also been shown that PPARs can induce transcription of acyl coenzyme A oxidase and cytochrome P450 through interaction with specific response elements.
Synonyms:	rabbit anti-Ppar alpha antibody, Pparα, Peroxisome proliferator-activated receptor alpha, PPAR-alpha, Nuclear receptor subfamily 1 group C member 1, Ppar-a, Nr1c1, Ppar
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	Ppara
Reactivity:	Human, Mouse
Immunogen Type:	Conjugated Peptide
Immunogen:	PPAR alpha Antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to a N-Terminal region near amino acids 1-25 of mouse PPAR alpha.
Purity/Specificity:	This affinity purified antibody is directed against mouse PPAR alpha protein. The product was affinity purified from monospecific antiserum by immunoaffinity purification. A BLAST analysis was used to suggest reactivity with this protein from mouse, rat, bovine, dog, golden hamster and boar sources based on 100% homology for the immunogen sequence. Cross reactivity with PPAR alpha protein from human, chimpanzee and rhesus monkey may also occur as this sequence shows 88% homology (16/18 identities) with the protein from these sources. Cross reactivity with PPAR alpha homologues from other sources has not been determined. No reactivity is expected against other subtypes of PPAR.
Relevant Links:	• GeneID - 19013 • NCBI - 31543500 • UniProtKB - P23204

Application Details

Tested Applications:	ELISA, IF, IHC, WB
Suggested Applications:	ChIP (Based on references)
Application Note:	Anti-PPAR alpha Antibody has been tested in ELISA, Western Blot, Immunohistochemistry, and Immunofluorescence. Expect a single band approximately 52 kDa in size corresponding to PPAR alpha by western blot in the appropriate tissue or cell lysate. A 1:200 dilution is suggested for Immunohistochemistry. Specific conditions for reactivity should be optimized by the end user.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:8,000 - 1:32,000
FC:	User Optimized
IHC:	1:100-1:300
WB:	1:500 - 1:2,000

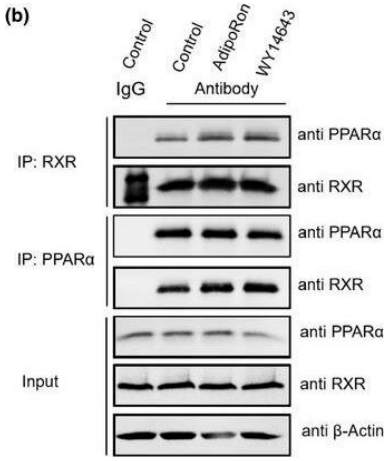
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

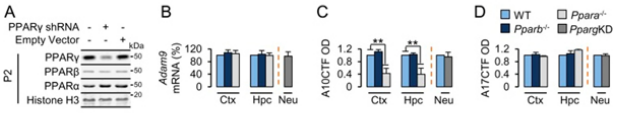
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



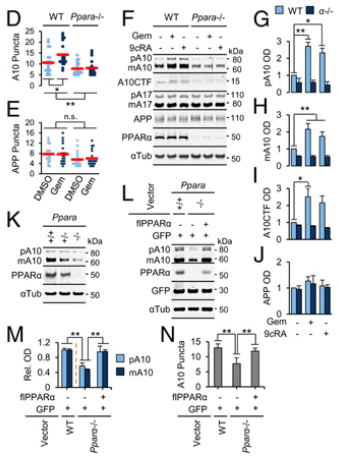
Western Blot

Adipopnectin regulates the expression of ADAM10 and Notch1 through PPARα and JNK pathway respectively. (a) Representative immunoblots and quantification of hippocampal protein levels after chronic ICV injection of AdipoRon, WY14643 (PPARα agonist) and GW6471 (PPARα antagonist) in 12-month-old mice, including Notch1 and ADAM10. Control, n = 6; AdipoRon, n = 6; WY14643, n = 7; AdipoRon + GW6471, n = 7. *p < 0.05, **p < 0.01, ***p < 0.001 compared with Control; #p < 0.05, ###p < 0.001 compared with AdipoRon treatment. (b) Co-immunoprecipitation results showing chronic ICV injection of AdipoRon or WY14643 enhances the interaction between PPARα and RXR. Control, n = 6; AdipoRon, n = 6; WY14643, n = 7. ***p < 0.001 compared with Control. (c) qPCR results showing chronic ICV injection of AdipoRon or WY14643 Induces the recruitment of RXR to the ADAM10 Promoter. Control, n = 6; AdipoRon, n = 7; WY14643, n = 7. ***p < 0.001 compared with Control. (d) Chronic ICV injection of AdipoRon upregulates the activity of JNK. Control, n = 6; AdipoRon, n = 6. ***p < 0.001. (e) Representative immunoblots and quantification of hippocampal protein levels after chronic ICV injection of AdipoRon, Vinblastine (JNK agonist) and SP600125 (JNK antagonist) in 12-month-old mice, including Notch1 and ADAM10. Control, n = 6; AdipoRon, n = 7; Vinblastine, n = 7; AdipoRon + SP600125, n = 7. **p < 0.01, ***p < 0.001 compared with Control; ###p < 0.001 compared with AdipoRon treatment. Data are presented as means ± SEM Figure provided by CiteAb. Source: Aging Cell, PMID: 34053165.



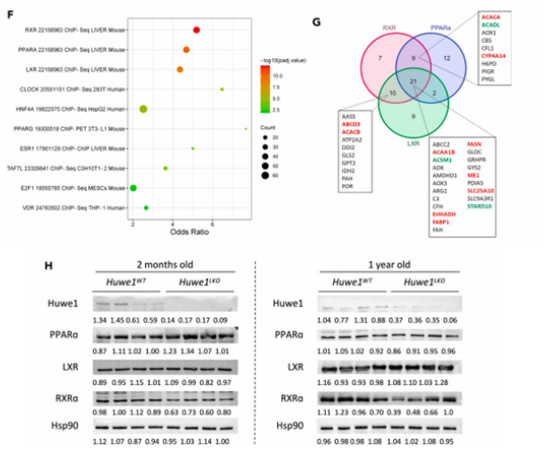
Western Blot

(A) Representative immunoblots of PPARγ, PPARβ, and PPARα in nuclear extracts prepared from 18DIV WT neurons transfected for 48 h with a PPARγ-shRNA plasmid (OriGene #TG320459) or empty vector (OriGene #TR30013). Histone H3 served as loading control. (B) Quantitative PCR results of Adam9 mRNA expression in the hippocampus and cortex of 4-mo-old WT, Ppara−/−, and Pparb−/− animals or in 18DIV WT neurons knocked down for PPARγ (PpargKD). Values are corrected for Gapdh and are expressed as percentage of WT. (C and D) Quantification of C-terminal-cleaved ADAM10 (A10CTF; C) and ADAM17 (A17CTF; D) membrane expression in the hippocampus and cortex of 4-mo-old WT, Ppara−/−, and Pparb−/− animals or in 18DIV PpargKD neurons. Representative immunoblots are presented in Fig. 1F. Values are corrected for β-tubulin, indicate the mean ± SEM relative to WT, and represent n = 3 or 4 for each condition. **P < 0.01, using two-way ANOVA. Ctx, cortex; Hpc, hippocampus; KD, knockdown; Neu, neurons; OD, relative optical density. SF1. PMID: 26080426



Western Blot

Representative immunoblots (F) and quantification of pA10 (G), mA10 (H), A10CTF (I), and APP (J) membrane expression in WT and Ppara−/− neurons treated with DMSO, 25 µM Gem, or 0.2 µM 9-cis-retinoic acid (9cRA) for 24 h. (K) Representative immunoblots of membrane-bound ADAM10 expression in hippocampi from 2-mo-old Ppara WT (+/+), heterozygous (+/−), and null (−/−) mice. (L and M) Representative immunoblots (L) and quantification (M) of pA10 and mA10 membrane expression in WT neurons transduced with GFP alone and in Ppara−/− neurons transduced with GFP alone or with flPPARα. (N) Quantification of surface ADAM10 expression in live-stained, unpermeabilized, 18DIV WT neurons transduced with GFP alone and in Ppara−/− neurons transduced with GFP alone or with flPPARα. The corresponding immunocytochemical images are presented in Fig. S2D. All values are corrected for β- or α-tubulin, indicate the mean ± SEM relative to control, and represent n = 3 or 4 for each treatment with the exception of E, F, and N, in which n ≥ 20. *P < 0.05 and **P < 0.01, using two-way (D, E, M) or one-way (N) ANOVA. (Scale bar, 5 µm.) n.s., not statistically significant; OD, relative optical density. Fig 2. PMID: 26080426



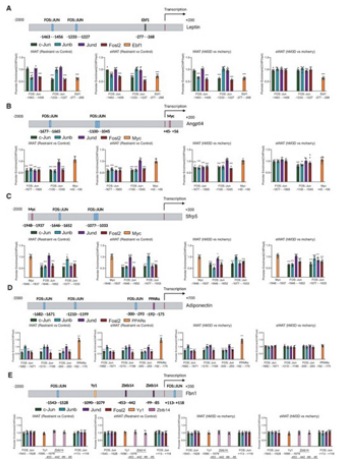
Western Blot

(F and G) ChEA analysis on all 127 differentially expressed proteins in Huwe1LKO livers revealed strong enrichment of RXR, PPARα, and LXR activity (F). Many of the differentially expressed proteins in Huwe1LKO livers are predicted to be regulated by RXR, PPARα, and/or LXR (G). Upregulated (Green) and downregulated (Red) proteins related to fatty acid metabolism are highlighted.

(H) Western blot was performed for the indicated proteins in livers isolated from 2-month-old and 1-year-old Huwe1WT and Huwe1LKO mice. Band densitometric values shown indicate Hsp90-normalized expression values relative to average expression of Huwe1WT samples.

Fig 3.

PMID: 38047073



ChIP

Binding of AP-1 proteins to the promoters of adipokines under chronic restraint stress or chronic activation of PVN neurons.

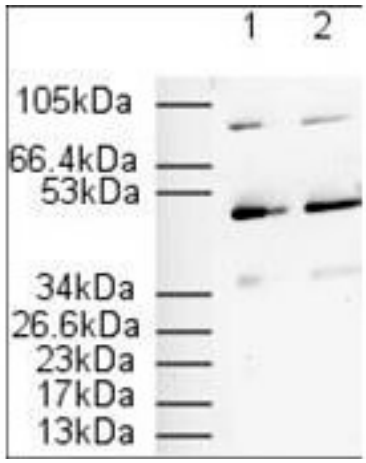
A–E

ChIP assays showing the binding of AP-1 family members (c-Jun, Junb, Jund and fosl2), Ebf1, PPARα, Yy1 and Myc to the promoters of adipokines (A, leptin; B, Angptl4; C, Sfrp5; D, adiponectin; E, Fbn1) under chronic stress or chronically activating PVN neurons.

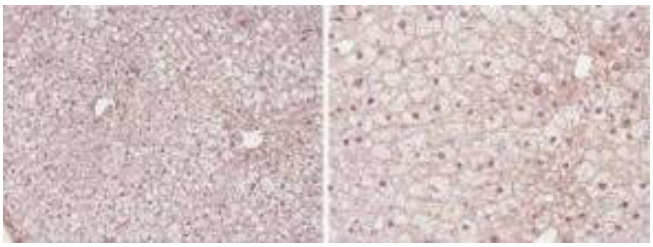
Data information: N = 8 for each group in (A–E).

Shapiro–Wilk test and F-test were used to test the normality and equal variance assumptions, respectively.

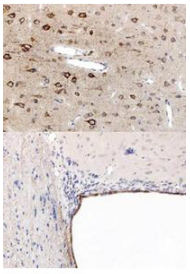
Two-tailed t-tests were performed to assess differences between two experimental groups with normally distributed data and equal variance. Two-tailed t-tests with Welch's correction were used when a two-sample comparison of means with unequal variances. For non-normally distributed data, Mann–Whitney U-tests were performed to compare two groups. $P < 0.05$ was considered statistically significant. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Data are presented as means ± SEM. Fig 12. PMID: 37870400



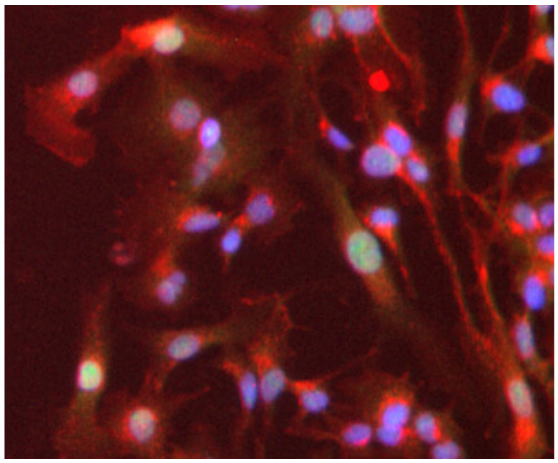
Western Blot
Affinity Purified Anti-PPAR alpha (N -terminal specific) (Rabbit) is shown to detect a 52 kDa band corresponding to PPAR alpha present in a 3T3 whole cell lysate. Approximately 20 µg of lysate was loaded per lane for SDS-PAGE. Detection occurred after using a 1:500 (lane 1) or 1:1000 (lane 2) dilution of antibody followed by 1:2000 dilution of HRP Goat-a-Rabbit IgG for visualization.



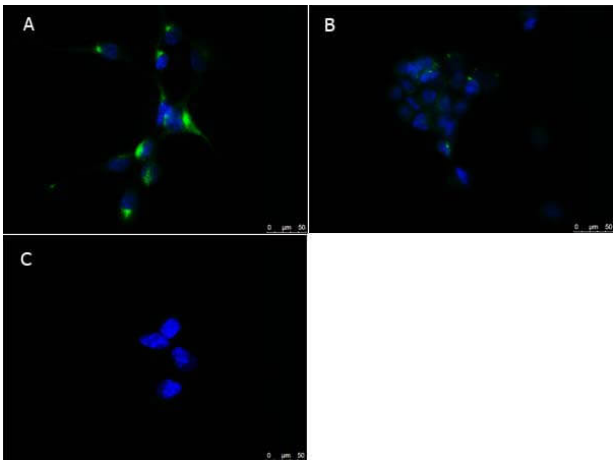
Immunohistochemistry
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) showing Rockland's PPAR alpha antibody staining of PPAR alpha protein in mouse liver tissue section (Formalin/PFA-fixed paraffin-embedded sections). Tissue underwent formaldehyde fixation before enzymatic antigen retrieval with 0.05% protease in PBS for 5 minutes. Sample was then blocked with 5% serum for 20 minutes at 20°C. The primary antibody was diluted 1:50 and incubated with sample in Tris plus 5% normal goat serum for 1 hour at 20°C. A Biotin conjugated goat polyclonal to rabbit IgG was used at dilution at 1:500 as secondary antibody. Images show nuclear staining in hepatocytes (perfusion-fixed mouse, 10 and 40x microscope magnification).



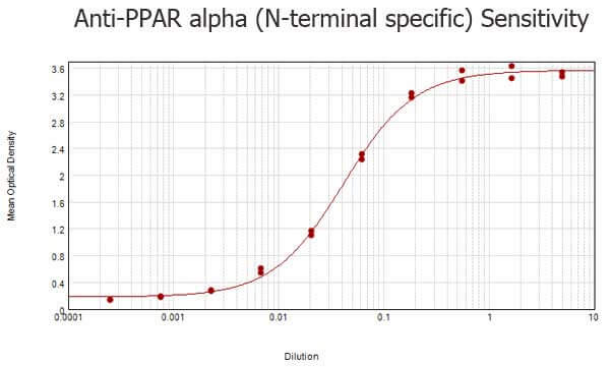
Immunohistochemistry
Immunohistochemistry using Rockland's anti-PPAR antibody, showing staining of PPAR alpha in rat brain sections, highlighting cytoplasmic staining in ependymal cells and neurons in frontal cortex. Bottom image shows subventricular zone (svz) of lateral ventrical (exit point of progenitor olfactory neurones); top image shows frontal cortex in the same section. Cytoplasmic staining is also observed in the corpus callosum (bottom image) and in dendritic fields of the cortex. Formalin/PFA-fixed paraffin-embedded sections of rat brain tissue were incubated with the primary antibody at 1:200 for 1 hour. Antigen retrieval was performed by heat induction in citrate buffer pH 6.0.



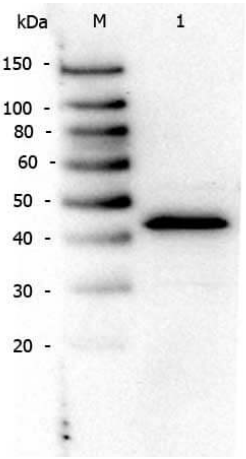
Immunofluorescence Microscopy
Immunofluorescence Microscopy of Rabbit anti-PPAR alpha antibody. Tissue: HepG2 cells. Fixation: 4% formaldehyde fixed (10 min). Antigen retrieval: not required. Primary antibody: PPAR alpha antibody at 1 µg/mL overnight at 4°C. Secondary antibody: Alexa Fluor® 488 goat anti-rabbit IgG (H+L) (green) used at a 1:1000, Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1:200 dilution for 1h for 45 min at RT. Localization: PPAR alpha is nuclear and occasionally cytoplasmic. Staining: PPAR alpha as green fluorescent signal with DAPI (blue) nuclear counterstain.



Immunofluorescence Microscopy
Immunofluorescence microscopy of Rabbit Anti-PPAR alpha (N-terminal specific) antibody using (A) Mouse NIH/3T3 or (B) Human HEK293 cells fixed with MeOH. (C) Secondary antibody only with NIH/3T3 cells. Anti-PPAR alpha antibody was used at 10 µg/mL, 1h at RT°. Secondary antibody: Anti-RABBIT IgG DyLight™ 488 Conjugated Preadsorbed (p/n 611-741-127) at 5 ug/ml for 1 h at RT. Staining: PPAR as green fluorescent signal with DAPI (blue) nuclear counterstain.

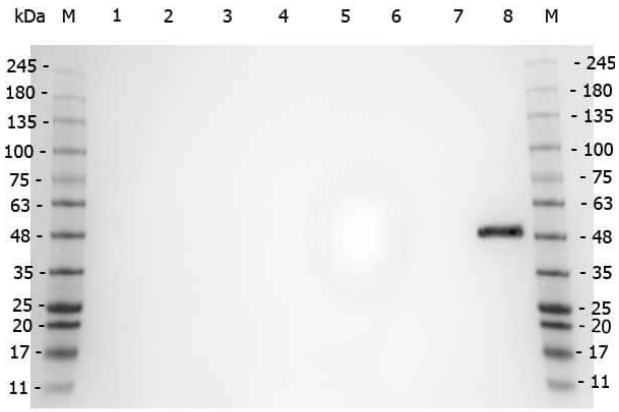


ELISA
ELISA results of purified Rabbit anti-PPAR Alpha (N-terminal specific) Antibody tested against BSA-conjugated peptide of immunizing peptide. Each well was coated in duplicate with 0.1µg of conjugate. The starting dilution of antibody was 5µg/ml and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using 3% fish gel, Goat anti-Rabbit IgG Antibody Peroxidase Conjugated (Min X Bv Ch Gt GP Ham Hs Hu Ms Rt & Sh Serum Proteins) (p/n 611-103-122) and TMB ELISA Peroxidase Substrate (p/n TMBE-1000).



Western Blot

Western Blot of Rabbit anti-PPAR Alpha (N-terminal Specific) antibody. Lane M: Prestained Molecular Weight Markers. Lane 1: NIH/3T3 (p/n W10-000-358). Load: 10 µg per lane. Primary antibody: PPAR Alpha (N-terminal specific) antibody at 1:1,000 for overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody (p/n 611-103-122) at 1:40,000 for 30 min at RT. Block: Blocking Buffer for Fluorescent Western Blotting (p/n MB-070) at RT for 30 min. Predicted/Observed size: ~50 kDa for PPAR Alpha.



Western Blot

Western Blot of Rabbit anti-PPAR Alpha (N-terminal specific) antibody. Marker: Opal Pre-stained ladder (p/n MB-210-0500). Lane 1: HEK293 lysate (p/n W09-000-365). Lane 2: HeLa Lysate (p/n W09-000-364). Lane 3: MCF-7 Lysate (p/n W09-000-360). Lane 4: Jurkat Lysate (p/n W09-000-370). Lane 5: A431 Lysate (p/n W09-000-361). Lane 6: LNCaP Lysate (p/n W09-001-GJ9). Lane 7: A-172 Lysate (p/n W09-001-GL5). Lane 8: NIH/3T3 Lysate (p/n W10-000-358). Load: 35 µg per lane. Primary antibody: PPAR Alpha (N-terminal specific) antibody at 1ug/mL overnight at 4°C. Secondary antibody: Peroxidase rabbit secondary antibody (p/n 611-103-122) at 1:30,000 for 60 min at RT. Blocking Buffer: 1% Casein-TTBS (p/n MB-082) for 30 min at RT. Predicted/Observed size: 52 kDa for PPAR Alpha.

References

- Feng WW et al. Hepatic Huwe1 loss protects mice from non-alcoholic fatty liver disease through lipid metabolic rewiring. *iScience.* (2023)
- Wang J et al. Role and mechanism of PVN-sympathetic-adipose circuit in depression and insulin resistance induced by chronic stress. *EMBO Rep.* (2023)
- You J et al. Role of Adiponectin-Notch pathway in cognitive dysfunction associated with depression and in the therapeutic effect of physical exercise. *Aging Cell.* (2021)
- Corbett GT et al. Activation of peroxisome proliferator-activated receptor α stimulates ADAM10-mediated proteolysis of APP. *Proc Natl Acad Sci USA.* (2015)

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