COSMO BIO CO., LTD.

Real Time PCR Primer Set for Adipose - mouse

Cat. No : PCRM2 (Japan) Cat. No.: PMC-PCRM2 (Outside Japan)

※Please read this manual carefully before use. This product is intended for research use only.

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This kit is a primer set optimized for mouse adipocytes for use in real-time PCR (SYBR Green method). The kit contains a total of 29 genes, selected from commonly used differentiation and metabolic markers in adipocyte and adipose tissue research, as well as 3 reference genes, combined into a single set. This kit is suitable for gene expression analysis using mouse primary adipocytes, 3T3-L1 cells, and mouse

Kit Components

Component (Gene names are indicated using official gene symbols.)	Volume	Number of vials	Storage Temperature
Reference gene primers (3 genes) • Actb • Gapdh • Hprt	1000 pmole each	1 vial each (Total: 32 vials)	Room Temperature Store at 4°C after reconstitution.
Target gene primers (29 genes) • Adipoq • Adrb3 • Cebpa • Cebpb • Cebpd • Fabp4（also known asaP2） • Il10 • Il1b • Il6 • Insr • Irs1 • Irs2 • Lep • Ccl2（also known as MCP1） • Serpine1 (also known asPAI-1) • Plin1 • Pparg • Rbp4 • Retn • Slc2a1 (also known asGlut-1) • Slc2a4（also known asGlut-4） • Tnf • Ucp1 • Ucp2 • Ucp3 • Ppargc1a（also known asPGC1A） • Lipe • Hsd11b1 • Prdm16	500 pmole each		

※Each gene-specific primer set contains equal amounts of forward (FW) and reverse (RV) primers (1,000 pmol or 500 pmol each).

※This kit is supplied as a lyophilized powder. Before use, reconstitute the product in sterile water or an appropriate buffer such as TE buffer.

※The gene primer design algorithm has been validated by amplification curve and melting curve analyses using LightCycler ST300 and LC480 (Roche Diagnostics) and DNA Engine Opticon 2 (MJ Research; now Bio-Rad Laboratories).
※Each gene-specific primer included in this kit can be used for RT-PCR applications.

Reference gene primer information

Gene name （Official Full Name）	Label	Accession Number	Amplicon Size	Tm value	
				FW Primer	RV Primer
actin, beta	Mouse_ACTB(F+R)	NM_007393	200	62.1	62.2
glyceraldehyde-3-phosphate dehydrogenase	Mouse_GAPDH(F+R)	NM_008084	241	62.0	62.2
hypoxanthine guanine phosphoribosyl transferase	Mouse_HPRT1(F+R)	NM_013556	195	62.0	61.5

Target gene primer information

Gene name （Official Full Name）	Label	Accession Number	Amplicon Size	Tm value	
				FW Primer	RV Primer
adiponectin, C1Q and collagen domain containing	Mouse_ADIPOQ(F+R)	NM_009605	203	61.0	59.8
adrenergic receptor,beta 3	Mouse_ADRB3(F+R)	NM_013462	202	62.2	62.0
CCAAT/enhancer binding protein (C/EBP), alpha	Mouse_CEBPA(F+R)	NM_007678	186	62.7	61.9
CCAAT/enhancer binding protein (C/EBP), beta	Mouse_CEBPB(F+R)	NM_009883	195	58.4	59.6
CCAAT/enhancer binding protein (C/EBP), delta	Mouse_CEBPD(F+R)	NM_007679	216	57.5	57.8
fatty acid binding protein 4, adipocyte	Mouse_FABP4(F+R)	NM_024406	194	59.3	59.9
interleukin 10	Mouse_IL10(F+R)	NM_010548	189	61.3	61.2
interleukin 1 beta	Mouse_IL1B(F+R)	NM_008361	208	58.1	60.4
interleukin 6	Mouse_IL6(F+R)	NM_031168	219	57.0	56.7
insulin receptor	Mouse_INSR(F+R)	NM_010568	194	61.5	61.8
insulin receptor substrate 1	Mouse_IRS1(F+R)	NM_010570	208	58.7	57.9
insulin receptor substrate 2	Mouse_IRS2(F+R)	NM_001081212	195	59.6	60.5
leptin	Mouse_LEP(F+R)	NM_008493	204	57.6	57.9
chemokine (C-C motif) ligand 2	Mouse_MCP1(F+R)	NM_011333	200	61.3	62.5
serine (or cysteine) peptidase inhibitor, clade E, member 1	Mouse_PAI1(F+R)	NM_008871	201	62.1	61.0
perilipin 1	Mouse_PLIN(F+R)	NM_175640	203	62.7	62.3
peroxisome proliferator activated receptor gamma	Mouse_PPARG(F+R)	NM_011146	205	62.1	61.7
retinol binding protein 4, plasma	Mouse_RBP4(F+R)	NM_011255	218	58.0	58.6
resistin	Mouse_RETN(F+R)	NM_022984	192	58.1	57.5

Gene Name （Official Full Name）	Label	Accession Number	Amplicon Size	Tm value	
				FW Primer	RV Primer
solute carrier family 2 (facilitated glucose transporter), member 1	Mouse_SLC2A1(F+R)	NM_011400	183	60.4	60.4
solute carrier family 2 (facilitated glucose transporter), member 4	Mouse_SLC2A4(F+R)	NM_009204	181	59.7	60.0
tumor necrosis factor	Mouse_TNF(F+R)	NM_013693	212	58.8	57.8
uncoupling protein 1 (mitochondrial, proton carrier)	Mouse_UCP1(F+R)	NM_009463	238	60.4	61.9
uncoupling protein 2 (mitochondrial, proton carrier)	Mouse_UCP2(F+R)	NM_011671	188	61.7	62.5
uncoupling protein 3 (mitochondrial, proton carrier)	Mouse_UCP3(F+R)	NM_009464	193	60.5	60.1
peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	Mouse_PGC1A(F+R)	NM_008904	198	62.2	62.1
lipase, hormone sensitive	Mouse_LIPE(F+R)	NM_001039507	209	61.0	61.8
hydroxysteroid 11-beta dehydrogenase 1	Mouse_HSD11B1(F+R)	NM_001044751	196	61.9	61.9
PR domain containing 16	Mouse_PRDM16(F+R)	NM_001177995	210	61.5	62.5

Reconstitution of gene primers

The amount per gene differs between the reference gene primers and the target gene primers. Please pay attention to the volume of solvent added during reconstitution.

1. The contents of each gene-specific primer tube are supplied in a dried form and may adhere to the inside of the tube cap.

2. Before opening the tube, centrifuge briefly to collect the contents at the bottom of the tube. After confirming the reaction conditions of your real-time PCR instrument, add an appropriate solvent (e.g., sterile water or TE buffer) according to the table below to achieve a concentration suitable for your experimental purpose, and dissolve the primers thoroughly.

For reference gene primers (1,000 pmol each per tube)

Volume to add	Final primer concentration
50 µL	20 µM each
100 µL	10 µM each
200 µL	5 µM each
1000 µL	1 µM each

Target gene primers (500 pmol/tube)

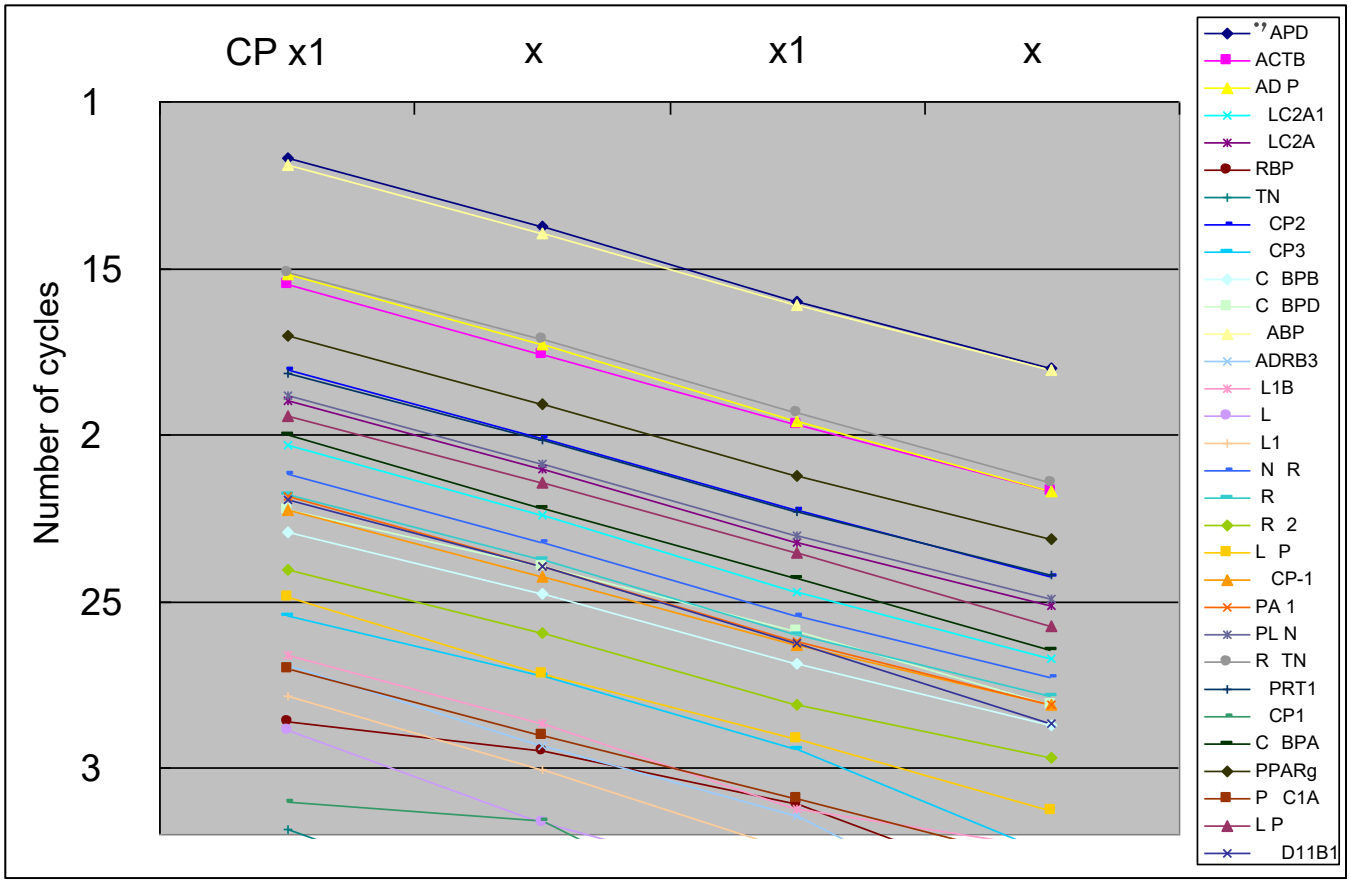
Volume to add	Final primer concentration
25 µL	20 µM each
50 µL	10 µM each
100 µL	5 µM each
500 µL	1 µM each

3. After reconstitution, store at 4 °C.

Specificity of gene primers

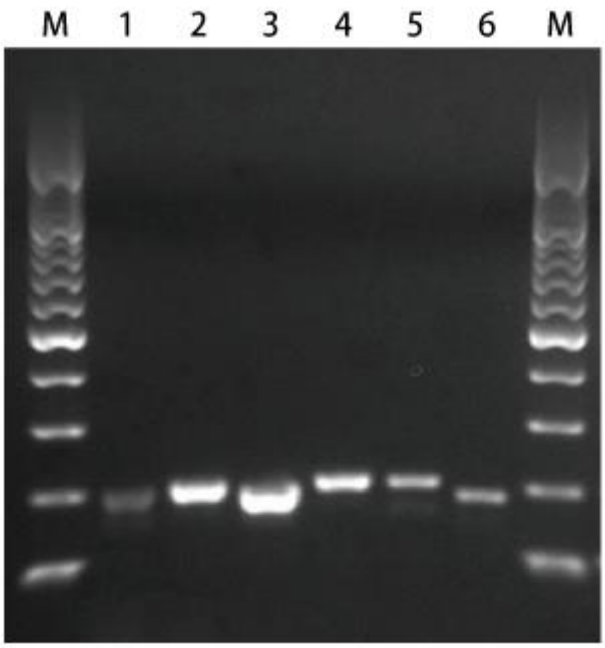
The gene-specific primers have been confirmed to exhibit appropriate amplification efficiency and specificity based on amplification curve and melting curve analyses performed using a real-time PCR instrument.

The figure below shows the results of analysis of serially diluted first-strand cDNA (1×, 4×, 16×, and 64× dilutions) prepared from 3T3-L1 cells, analyzed using LightCycler ST300 and LC480 (Roche Diagnostics).



※The figure is shown as an example only, and results may vary depending on experimental conditions.

RT-PCR analysis example using 3T3-L1 cells



1 : Hprt
2 : Actb
3 : Fabp4
4 : Pparg
5 : Adipoq
6 : Slc2a4
M : 100bp Ladder

This kit has been designed to produce PCR amplicon sizes suitable for detection by conventional methods, and can therefore also be used for standard RT-PCR.

Note:
Because the amount of PCR product depends on the sample, PCR reagents, and instrument, optimization by the user is required.

The figure below shows an electrophoretic analysis on an agarose gel of RT-PCR products amplified for 25 cycles, using first-strand cDNA prepared from 3T3-L1 cells as the template.

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