Invitrogen
life technologies

Mouse/Rat GAPDH—Certified LUX™ Primer Set

Cat. Nos.	Size:
100M-01 (FAM labeled)	**100 µl × 2**
100M-02 (JOE labeled)	**100 µl × 2**
Conc: 10 µM	**Store at -20°C** (non-frost-free freezer)

LUX™ Primers

LUX™ primers are a sensitive and efficient method for performing real-time (quantitative) PCR and RT-PCR. Each LUX™ primer pair includes a fluorogenic primer and a corresponding unlabeled primer, which have been designed to amplify and detect a specific gene of interest. The hairpin secondary structure of the fluorogenic primer quenches the attached fluorophore (FAM or JOE). When this primer is incorporated into double-stranded PCR product, the fluorophore is dequenched and the signal increases by up to 10-fold. LUX™ primers combine high specificity with multiplexing and melting curve capability, have a broad dynamic range of 7–8 orders, and are compatible with most real-time PCR instruments.

Certified LUX™ Primer Sets for Housekeeping Genes

Certified LUX™ Primer Sets for Housekeeping Genes are predesigned primer sets for genes that are commonly used as internal controls for normalizing real-time RT-PCR experiments. These primer sets have been optimized and functionally validated to provide accurate, reproducible results using standard LUX™ protocols, and are supplied in a ready-to-use format.

Certified LUX™ Primer Sets contain a vial of labeled primer and a vial of unlabeled primer, each at 10 µM concentration. Volumes are sufficient for 100 50-µl reactions or 250 20-µl reactions. For real-time PCR and RT-PCR protocols using LUX™ primers, visit the LUX™ Web site at www.invitrogen.com/lux.

Primer	Direction	Amount	Conc
Labeled (FAM or JOE)	Forward	100 µl	10 µM
Unlabeled	Reverse	100 µl	10 µM

Part No. 100M.pps Rev. date: 05/09/03

Shipping and Storage

Primers are supplied in TE buffer and should be stored at -20°C in the dark in a non-frost-free freezer.

GAPDH

GAPDH (glyceraldehyde-3-phosphate dehydrogenase) catalyzes an important energy-yielding step in carbohydrate metabolism, the reversible oxidative phosphorylation of glyceraldehyde-3-phosphate in the presence of inorganic phosphate and nicotinamide adenine dinucleotide (NAD). The enzyme is thought to be a tetramer of identical chains.

Database information for GAPDH is provided in the table below:

Gene	Species	GenBank® Accession #	Entrez® Ref Seq #	OMIM™ #
GAPDH	*Mus musculus**	M32599	NM_008084	138400

*Primer set has been validated to work with both mouse and rat genes.

OMIM™ database: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM

Entrez® database: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide

Primer Specifications

The Mouse/Rat GAPDH Certified LUX™ Primer Set amplifies the region of GAPDH coding sequence that spans the exon junction 4/5.

Label	CDS Location	Amplicon Melting Temp**	PCR Product Size Range
JOE or FAM	Exons 4/5	T_m = 85°C	151–200 bp

**Note that this is the T_m of the amplicon, not the primers. T_m is approximate and dependent on experimental conditions.

Dye Information

Each fluorogenic LUX™ primer is labeled with one of two reporter dyes:

Dye	Wavelength	
	Excitation	Emission
FAM (6-carboxy-fluorescein)	490 nm	520 nm
JOE (6-carboxy-4', 5'-dichloro-2', 7'-dimethoxy-fluorescein)	520 nm	550 nm

Protocols

Primers are supplied at 10 µM concentration. Use 1 µl of each primer (labeled and unlabeled) per 50 µl reaction, or 0.4 µl per 20 µl reaction.

Refer to the LUX™ Primers manual for detailed protocols for performing real-time PCR and RT-PCR. The manual can be downloaded at www.invitrogen.com/lux.

Note: We strongly recommend DNase I digestion of RNA samples prior to amplification with LUX™ gene-specific primers. See the LUX™ Primers manual for more information.

Product Qualification

Certified LUX™ Primer Sets are designed to discriminate between messages and known pseudogenes/different isoforms. Mouse/rat primer sets have been validated to work with both mouse and rat genes. Performance is functionally validated using a dilution series in a two-step real-time RT-PCR with mouse NIH 3T3 RNA. The amplification efficiency based on the slope of the resulting standard curve is greater than 90%.

Limited Use Label License No. 114: LUX™ Fluorogenic Primer

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