



Human PGK1—Certified LUX™ Primer Set

Cat. Nos.	Size:
109H-01 (FAM labeled)	100 µl × 2
109H-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.

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

Part No. 109H.pps

Rev. date: 05/10/03

This product is distributed for laboratory research only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

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.

PGK1

Phosphoglycerate kinase 1 (PGK1), also known as ATP:3-phosphoglycerate 1-phosphotransferase, is a major enzyme in glycolysis that catalyzes the reversible conversion of 1,3-diphosphoglycerate to 3-phosphoglycerate, generating one molecule of ATP.

Database information for PGK1 is provided in the table below:

<u>Gene</u>	<u>Species</u>	<u>GenBank[®] Accession #</u>	<u>Entrez[®] Ref Seq #</u>	<u>OMIM[™] #</u>
PGK1	<i>Homo sapiens</i>	V00572	NM_000291	311800

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 Human PGK1 Certified LUX[™] Primer Set amplifies a region in the fourth quarter of the PGK1 coding sequence.

<u>Label</u>	<u>CDS Location</u>	<u>Amplicon Melting Temp^{**}</u>	<u>PCR Product Size Range</u>
JOE or FAM	n/a	T _m = 82°C	50–100 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:

<u>Dye</u>	<u>Wavelength</u>	
	<u>Excitation</u>	<u>Emission</u>
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. Performance is functionally validated using a dilution series in a two-step real-time RT-PCR with total HeLa 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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