

ISOQX® LAMP pH Sensitive Dyestuff Kit

Cat. no. LM701204

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Product Information

Cat. No.	Product name	Pack size
LM701204	ISOQX® LAMP pH Sensitive Dyestuff Kit	100 T
		1,000 T
		10,000 T

Please state the required pack size when ordering.

Kit Contents

Component No.	Component name	100 T	1,000 T	10,000 T
LM701204-A	2.5× pH Sensitive Reaction Buffer	1 mL	10 mL	100 mL
LM701204-B	Glycerol-free Bst Enzyme	50 µL	500 µL	5 mL

Storage

Part I: Component LM701204-A (2.5× pH Sensitive Reaction Buffer) is shipped on dry ice and stored at –20 °C. Shelf life: 6 months.

Part II: Component LM701204-B (glycerol-free Bst Enzyme) is shipped with ice packs and stored at 4 °C. Shelf life: 6 months.

Introduction

This product integrates a LAMP visible-light dye into the buffer. The buffer contains dATP, dCTP, dGTP, dUTP and other components required for amplification by Bst II DNA Polymerase, and also contains a pH-sensitive indicator.

The kit includes glycerol-free Bst II DNA Polymerase, UDGase and other components, and can be used to prepare lyophilised reaction systems. It also reduces pipetting steps and operating errors, greatly suppresses false-positive reactions, and improves detection accuracy.

The kit provides high amplification efficiency and sensitivity. Amplification results can be judged by the naked eye: positive reaction wells are orange-yellow, while negative reaction wells are magenta, giving a strong visual contrast.

Features

1. Visible-light pH-sensitive dye integrated directly into the buffer — results can be read by eye without instrumentation.
2. Glycerol-free Bst II DNA Polymerase and UDGase included — compatible with lyophilised reaction system preparation.
3. Fewer pipetting steps and reduced operating errors — greatly suppresses false-positive reactions and improves detection accuracy.
4. High amplification efficiency and sensitivity, with strong visual contrast between positive (orange-yellow) and negative (magenta) results.

Notes Please be sure to read this precaution before using the kit.

1. Read the instruction manual carefully before using this reagent.
2. Standardised procedures should be followed throughout the experiment, including preparation of the reaction system, sample processing and sample loading.
3. For your safety and health, wear a laboratory coat and disposable gloves during operation.

Protocol

A. Preparation of Test Samples

Samples after nucleic acid extraction: use directly as the template and add to the reaction system.

Boiling method: place the clinically collected swab sample directly into water, then heat it in a 95 °C water bath or metal bath for 5 min to lyse the sample and release nucleic acids. Mix well and use as the template for the reaction system.

- a. Do not use strongly acidic or strongly alkaline nucleic acid release reagents for sample processing. If such reagents must be used, adjust the sample pH to 8.0 after processing.
- b. Avoid introducing high-concentration buffer systems such as Tris-HCl in the added sample, as this may prevent the pH indicator from changing colour and affect interpretation of the results.

B. Reaction System (25 µL)

Take Component A out of –20 °C storage. After it has thawed, mix by inversion and briefly centrifuge to ensure that all liquid collects at the bottom of the tube. Prepare the following reaction system according to the number of samples to be tested. Negative and positive controls are usually required.

Component	Volume per reaction (µL)	Final concentration
2.5× pH Sensitive Reaction Buffer	10	1×
Bst Enzyme	0.5	0.32 U/µL
10× Primers	2.5	1×
Template	3	10 ng–1 µg
ddH ₂ O	add to 25	—

- a. For the negative control, it is recommended to include a blank control without template and an NTC control, generally using water.
- b. 10× Primers: 16 µM FIP/BIP, 2 µM F3/B3, and 4 µM each of Loop F/B.
- c. The template input volume can be adjusted within 1–8 µL.

C. Sample Loading

1. If the reaction is performed in a water bath, add 20 µL mineral oil (not provided) to each tube to prevent evaporation and avoid errors in the results. If the reaction is performed in a PCR instrument, mineral oil is not required; enable the heated lid.
2. To avoid contamination, users are advised to prepare components in a clean bench and add templates in a fume hood in another room to prevent false-positive results.
3. Because this reagent uses a pH-indicator method, the buffer capacity of the reagent is weak. The reagent should not be exposed to air for a long time; otherwise it may absorb carbon dioxide from the air and become acidic, causing the reagent colour to fade and affecting result interpretation.

D. Reaction Conditions

Temperature	Time	Purpose
25-37 °C	2-5 min	Degradation of U-containing templates
60-65 °C	30 min	Isothermal amplification
85 °C	5 min	Inactivation

a. The enzymes used in this kit are highly temperature-sensitive. Operation with a PCR instrument is strongly recommended. If a water bath is used, preheat it to the specified temperature before starting the reaction. A temperature deviation greater than 2 °C will reduce amplification efficiency and may prevent a positive reaction from changing from magenta to orange-yellow within the time specified in the manual.

b. The reaction temperature should be adjusted according to the T_m values of the primers.

c. The reaction time depends on template concentration. Low-concentration templates may require up to 60 min to produce an obvious reaction.

E. Result Interpretation

After 30 min of reaction, immediately remove the reaction tubes. After cooling to room temperature, observe them under good lighting conditions; using white paper as the background is recommended.

If the reaction solution is magenta, the result is negative. If the reaction solution is orange-yellow, the result is positive.

Note: the reaction time must be timed accurately. False-positive results may occur if the reaction exceeds the time specified in the manual. Do not open the reaction tubes, as this may cause contamination and affect subsequent experiments.