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Detection of Klebsiella pneumoniae and closely related species by real-time PCR with the ZKIR system

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1 *Works for me* dx.doi.org/10.17504/protocols.io.6gvhbw6

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ABSTRACT

Klebsiella pneumoniae (Kp) is of growing public health concern due to the emergence of multidrug-resistant and virulent strains. Taxonomically, Kp includes seven phylogroups, with Kp1 (*K. pneumoniae sensu stricto*) being medically prominent. Kp can be present in environmental sources such as soils and vegetation, which could act as reservoirs of animal and human infections. However, the current lack of screening methods to detect Kp in complex matrices limits research on Kp ecology.

We designed a novel SYBR green real-time PCR assay, named the ZKIR assay, that targets *Klebsiella pneumoniae* and closely related species (phylogroups Kp1 to Kp7).

Based on 48 Kp representing its phylogenetic breadth, and on 88 non-Kp strains, the ZKIR assay detected all Kp and was totally specific for Kp, as no false positive was found.

We also tested this method on spiked soil microcosms after a 24 h enrichment step (in Lysogeny Broth supplemented with ampicillin 10 mg/l) and a short sample treatment. We showed that this procedure was sensitive enough to detect one single bacterium in 5 g of soil.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Unpublished yet

Detection of Klebsiella pneumoniae Complex members by real time PCR_protocols.io.pdf

MATERIALS

NAME	CATALOG #	VENDOR
Takyon ROX SYBR MasterMix 2X Blue dTTP	View	
T4 bacteriophage Gene 32 product	SKU 11TGP32100	MP Biomedicals

MATERIALS TEXT

Primers sequences:

*ZKIR*_F : 5' CTAAAACCGCCATGTCCGAT 3'
*ZKIR*_R : 5' TTCCGAAAATGAGACACTTAGA 3'

Primers were synthetized by Eurogentec.

BEFORE STARTING

Takyon™ qPCR Kits for SYBR® assays containing ROX passive reference is used on the following thermocyclers: ABI Prism® 5700, ABI Prism® 7000, ABI Prism® 7300, ABI Prism® 7700, ABI Prism® 7900 & FAST 7900, ABI Step One & Step One Plus.
For other thermocyclers, see https://secure.eurogentec.com/egt/files/FileBrowse/Brochures/PCR%20-%20qPCR/Eurogentec-pcr-qpcr.pdf

1 Sample preparation

1.1 Sample enrichment step:

Soil samples (10 g) are enriched in 90 ml of LB (Lysogeny Broth: 5 g yeast extract, 5 g sodium chloride, 10 g tryptone for 1 liter) supplemented with ampicillin 10 mg/l for 24 h at 30°C.
Food samples (25g) such as salad and chicken are enriched in 225 ml of BPW (Buffered Peptone Water) for 24 h at 37°C.

1.2 Sample treatment step:

500 µl of enrichment is centrifuged 5 min at 5 800 g and washed twice with sterile water before boiling for 10 min. Boiled suspensions are then diluted at 1/10 and 1/100 for qPCR. Crude and diluted DNAs are tested with the ZKIR assay.

2 qPCR mix preparation (final volume 20 µl)

Mix reagents	Volume per well (µl)	Final concentration
Takyon™ ROX SYBR® MasterMix 2X	10	
Forward (3 µM)	2	300 nM
Reverse (3 µM)	2	300 nM
T4 gp32 (optional)	0.5	12.5 µg/ml
PCR grade water	QS 17.5 µl	
DNA template	2.5	

3

Thermocycler settings

	Temperature (°C)	Time (min.)	
Holding stage	95.0	03:00	Enzyme activation
Cycling stage	95.0	00:10	Denaturation
	60.0	01:00	Annealing (data collection)
Melt Curve stage	95.0	00:15	Dissociation stage Program Step and Hold T° increment +0.3°C
	60.0	01:00	
	95.0	00:15	

Thermocycler settings

4 ***Positive and negative controls:***

Negative control: PCR grade water
Positive control: *Kp*1 DNA diluted to 1 ng per µl (Strain ATCC13883T)

5 Melt curve peak established using the ZKIR assay with serial dilutions of K. pneumoniae ATCC13883T. Melting temperature was around 80.2 °C.