BactoReal® Kit
Leptospira spp. Multiplex (16S+LipL32)

For veterinary use only

BactoReal® Kit *Leptospira* spp. Multiplex (16S+LipL32)

Order no.	Reactions	Pathogen	Internal positive control
DVEB00713	100	FAM + VIC/HEX channel	Cy5 channel
DVEB00753	50	FAM + VIC/HEX channel	Cy5 channel

Kit contents:

- LipL32 detection assay for pathogenic *Leptospira* spp.
- 16S rDNA detection assay for pathogenic and intermediately pathogenic *Leptospira* spp.
- Detection assay for internal positive control (control of amplification)
- DNA reaction mix (contains uracil-N glycosylase, UNG)
- Positive controls for both *Leptospira* detection assays
- Water

Background: The spirochaetal *Leptospira* genus consists of pathogenic species (*L. interrogans, L. noguchii, L. weilii, L. kirschneri, L. alexanderi, L. borgpetersenii, L. santarosai, L. kmetyi, Leptospira* genomospecies 1), of intermediately pathogenic species (*L. inadai, L. fainei, L. broomii, L. licerasiae, L. wolffii*) and of non- pathogenic species (*L. biflexa, L. meyeri, L. wolbachii, Leptospira* genomospecies 3, 4 and 5). Members of *Leptospira* can also be grouped into serovars. Currently over 200 serovars are recognized in the genus *Leptospira* and a few serovars are found in more than one species of *Leptospira*. Non-pathogenic species are saprophytes and can grow outside the host animal, while pathogenic *Leptospira* species cause leptospirosis. They affect many mammalian species, including humans. Animals may become unapparent carriers, and shedding of leptospires, primarily in the urine, serves as a source of infection for other animals and humans. The clinical signs associated with leptospirosis are variable and depend on the infecting serovar and the susceptibility of the animal. Leptospires are present in the blood during the first 5 to 10 days after onset of the disease. Only pathogenic *Leptospira* species express an outer membrane lipoprotein encoded by the LipL32 gene.

Description: BactoReal® Kit *Leptospira* spp. Multiplex (16S+LipL32) is based on the detection and differentiation of the LipL32 gene of pathogenic *Leptospira* species (detection in FAM channel) as well as of the 16S rDNA gene of both intermediately pathogenic and pathogenic *Leptospira* species (detection in VIC/HEX channel) using multiplex real-time PCR. It facilitates the rapid and sensitive detection of DNA of *Leptospira* species from samples purified from blood, urine or kidney tissue (e.g. with the QIAamp DNA Mini Kit).

PCR-platforms: BactoReal® Kit *Leptospira* spp. Multiplex (16S+LipL32) is developed and validated for the ABI PRISM® 7500 instrument (Life Technologies), LightCycler® 480 (Roche) and Mx3005P® QPCR System (Agilent), but is also suitable for other real-time PCR instruments.

Sensitivity and specificity: BactoReal® Kit *Leptospira* spp. Multiplex (16S+LipL32) has an analytical sensitivity of 10 target copies/PCR reaction. The limit of detection (LoD95 = smallest number of copies of target RNA which can be detected in 95% of cases) is 20 target copies/reaction and was determined by several replicates around the detection limit. It was tested with 13 pathogenic and 3 non-pathogenic *Leptospira* serovars and 2 *Leptonema* species.

References:

- Levett, P.N. 2001. Leptospirosis. Clin. Microbiol. Rev. 14:296–326.
- Haake DA, Chao G, Zuerner RL, Barnett JK, Barnett D, Mazel M, Matsunaga J, Levett PN, Bolin CA. 2000. The leptospiral major outer membrane protein LipL32 is a lipoprotein expressed during mammalian infection. Infect. Immun. 68: 2276–2285.

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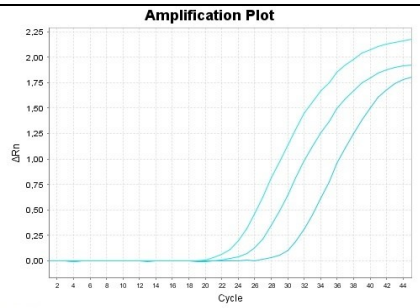
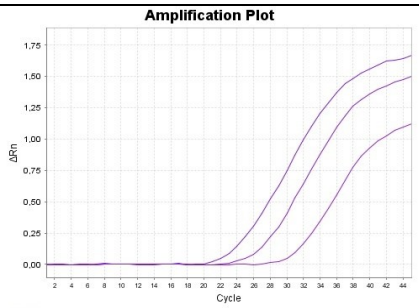
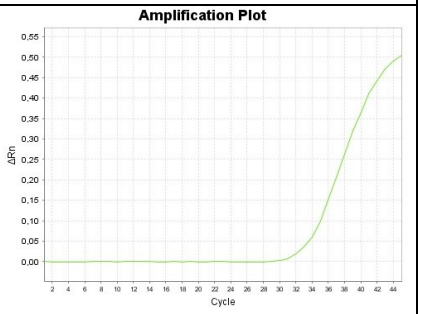
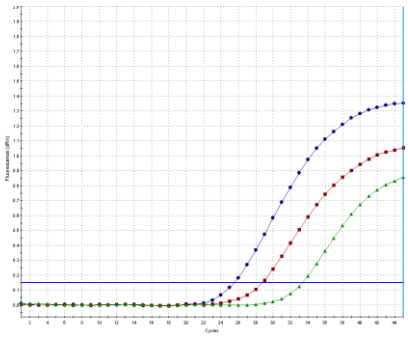
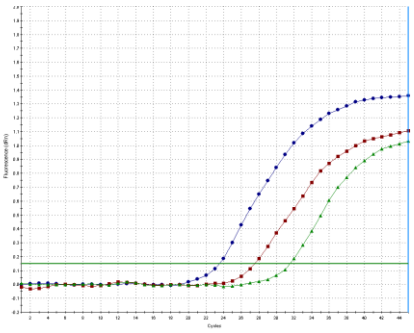
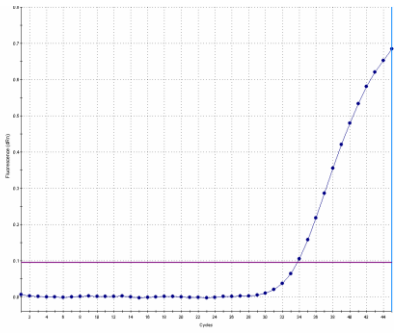
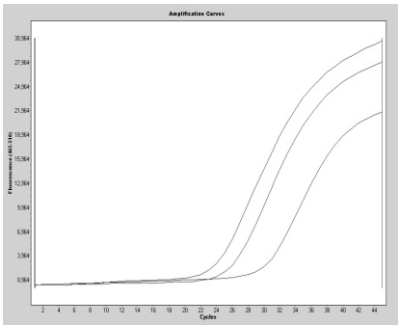
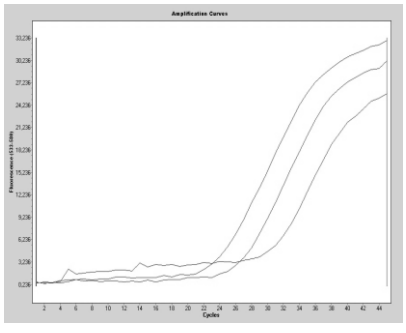
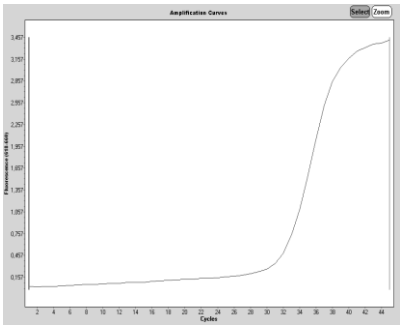
Detection of **pathogenic *Leptospira* spp.**	Detection of **pathogenic or intermediately pathogenic *Leptospira* spp.**	Detection of internal positive control **CR-3**
		
ABI Prism® 7500: FAM channel, 530 nm 1:10 serial dilution of *Leptospira* DNA	**ABI Prism® 7500:** VIC channel, 554 nm 1:10 serial dilution of *Leptospira* DNA	**ABI Prism® 7500:** Cy5 channel, 667 nm Internal positive control
		
Mx3005P®: FAM channel 1:10 serial dilution of *Leptospira* DNA	**Mx3005P®:** HEX channel 1:10 serial dilution of *Leptospira* DNA	**Mx3005P®:** CY5 channel Internal positive control
		
LightCycler® 480: FAM channel 1:10 serial dilution of *Leptospira* DNA	**LightCycler® 480:** VIC / HEX / Yellow555 channel 1:10 serial dilution of *Leptospira* DNA	**LightCycler® 480:** Cy5 channel Internal positive control

BactoReal®, MycoReal, ParoReal and ViroReal® Kits run with the same thermal cycling conditions. RNA and DNA material can be analysed in one PCR run.

For further information on our products please visit our homepage (www.ingenetix.com)

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