Application Note

Extraction of bacterial and fungal DNA with the InviSorb® Spin Soil DNA Kit

Soil samples can be very diverse, depending on their composition of mineral components and organic substances. Organic substances are soil organisms, such as bacteria and fungi, or degradation products of plant and animal origin. One method of analyzing the composition and fertility of soil samples is microbiome profiling by next-generation sequencing, in which the proportion and occurrence of bacteria and fungi in particular are characterized. For the extraction of microbial DNA a specific method is essential, because potential inhibitors of downstream reactions, especially humic acid, have to be removed from the sample material.
Here, DNA extraction of microorganisms from different soil samples was performed with the **InviSorb® Spin Soil DNA Kit** as well as with a soil-specific kit from another manufacturer. Subsequently, PCR experiments specific for bacterial and fungal DNA were performed with all extracts. The **InviSorb® Spin Soil DNA Kit** was found to outperform the competitor kit by far, as all PCR reactions could be successfully conducted, demonstrating efficient removal of inhibitors from soil samples with the **InviSorb® Spin Soil DNA Kit**.

EXPERIMENTAL SETUP

Soil samples were collected at different locations in north-east Germany (lake mud, garden soil, sand) or purchased at a local store (commercial potting soil) and were stored at -20°C until extraction. 200 mg each were extracted with the **InviSorb® Spin Soil DNA Kit** and an additional soil specific extraction kit from another manufacturer (competitor M). To evaluate the presence of bacteria and fungi the PCR kits Bacterial 16S rDNA PCR Kit Fast (800) (TaKaRa, Cat.# RR182A) and Fungal rDNA (ITS1) PCR Kit Fast (TaKaRa, Cat.# RR183A) were used according to the manufacturer's instructions. To visualize PCR products agarose gel electrophoresis was done.

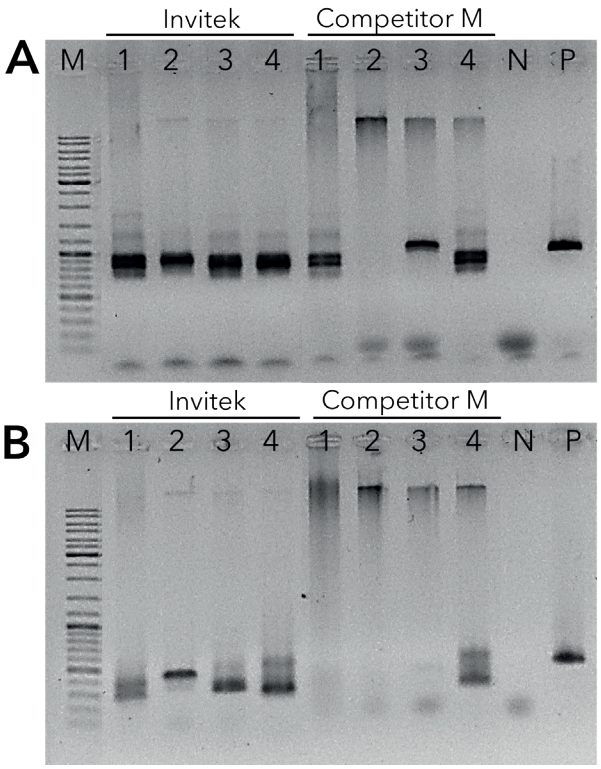
Fig. 1: InviSorb® product box

RESULTS & DISCUSSION

The PCR kits used in this experiment contain primers that amplify specific regions of ribosomal DNA used for identification of different bacterial and fungal species. Figure 2 shows the PCR result for the amplification of bacterial 16S rDNA (Fig. 2 A) and fungal ITS1 rDNA (Fig. 2 B). When using the **InviSorb® Spin Soil DNA Kit** for sample extraction, detection of fungal as well as bacterial DNA was successful for all soil types analyzed. In contrast, detection of fugal DNA failed in three out of four samples, when the competitor kit was used, and detection of bacterial DNA was confirmed only in three out of four cases.

The results show that inhibitors can be removed highly efficiently with the **InviSorb® Spin Soil DNA Kit** regardless of the soil type analyzed. PCR is not inhibited and succeeds very well even for organisms that are difficult to lyse, such as fungi. When using the competitor kit, a poor result is shown for the amplification of fungal DNA, which was only successful for sandy soil. In sandy soils, the proportion of organic components is low, so that comparatively few substances, such as humic acids, must be removed from the sample material.

Furthermore, the gel image shows that the band intensities of amplicons from the **InviSorb® Spin Soil DNA Kit** are higher than for the PCR results generated with the competitor kit, which indicates an overall higher DNA yield and quality in the eluates of samples extracted with the **InviSorb® Spin Soil DNA Kit**.



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No.	Soil type
1	Lake mud
2	Garden soil
3	Potting soil
4	Sand

Fig. 2: **PCR results for the detection of bacterial and fungal DNA in various soil samples.** A: PCR result for Bacterial 16S rDNA PCR Kit Fast (TaKaRa). For extraction with the Invitek kit, PCR was successful for all soil types, for extraction with competitor M for three of the samples. B: PCR result for Fungal rDNA (ITS1) PCR Kit Fast (TaKaRa). For extraction with the Invitek kit, PCR was successful for all soil types, for extraction with competitor M PCR worked for one sample only. N: negative control, P: positive control, M: marker. C: Legend of the soil samples used for extraction and PCR.

CONCLUSIONS

- The **InviSorb® Spin Soil DNA Kit** is suitable for the extraction of both bacterial and fungal DNA from soil samples, demonstrating that DNA can be successfully extracted from microorganisms that are difficult to lyse.
- The **InviSorb® Spin Soil DNA Kit** efficiently removes inhibitors from a variety of different soil samples so that no inhibition of downstream reactions occurs. The performance of the kit significantly exceeds that of the competitor kit.
- The high quantity of PCR products generated with the eluates of the **InviSorb® Spin Soil DNA Kit** shows that the purified DNA has a high yield and quality.

INVITEK DIAGNOSTICS GERMANY
Invitek Molecular GmbH
Robert-Roessle-Str. 10
13125 Berlin, Germany

Phone: +49 30 9489 2908

info@invitek.com

INVITEK DIAGNOSTICS PORTUGAL
ALS Life Sciences, SA
Zona Industrial de Tondela, ZIM II, Lote 6
3460-070 Tondela, Portugal

Phone: +351 232 817 817