PIKA SEEDEX MAX

DNA ISOLATION KIT FOR GRAIN – LARGE VOLUME

Art. No: 2019-20
(20 samples)

PIKA SEEDEX MAX is intended for the isolation of highly pure DNA from up to 20 grams milled cereals like grain and malt samples.

The kit can be applied both for the detection of DNA from cereals as well as from microbiological contaminants, e.g. moulds like *Fusarium*. The sample treatment results in the effective removement of potentially DNA degrading material as well as of PCR inhibitors. Extracted DNA may be used in several downstream applications as PCR.

It is mandatory to use only the provided spin filters and to dilute buffers LPT 10 x conc., WPF1 and WP2 prior to use (ref. page 2 "Before you start").

Store all components at room temperature.

Description	Volume	amount
Microspin column 2	-	20
Receiver tubes 2.0 ml	-	20
Receiver tubes 1.5 ml	-	20
LPT (10 x conc.) makes 1,233 ml each	123 ml	3
BPF (binding buffer)	6.5 ml	1
WPF1 (1,625 x conc.) makes 10.5 ml incl. EtOH	6.5 ml	1
WPF2 (5 x conc.) makes 21.25 ml incl. EtOH	4.25 ml	1
EP (elution buffer)	2.5 ml	1

Table 1: Contents

Additionally required instruments:

- Waterbath and stirrer (mash bath) or thermomixer (65 °C ± 5 °C)
- Vortexer
- Centrifuges for Falcon tubes and reagent tubes

Additionally required material and reagents:

- Sterile water
- ethanol, 96%
- iso-propanol
- Single use filter units for water solutions, 0,45 µm pore size, one per sample
- Syringes, ≥ 5 ml, one per sample
- 3 sterile bottles with min. Volume of 1,233 ml

Before you start

Safety Information

During the work with chemicals always wear a lab coat, disposable gloves and protective goggles.

BPF binding buffer and WPF1 washing buffer contain guanidine hydrochloride which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water.

Risk and safety phrases applying to BPF binding buffer and WPF1 washing buffer: Both buffers are containing guanidine hydrochloride which is harmful and irritant. Risk and safety phrases: R22-36 and R38, S 13, S26, S36 and S46 apply.

Buffer preparation

- **LPT** is delivered as a concentrate and has to be diluted 1:10 before first use:

  For each bottle, mix 1.110 ml of sterile water with the total content of the delivered bottle LPT 10 x conc. (123 ml each) in a sterile bottle to make the solution ready for use.

- Both washing buffers are delivered as concentrates and have to be diluted prior to use as follows:

  a. **WPF1**: Add 4,0 ml ethanol to total volume of bottle

  b. **WPF2**: Add 17 ml ethanol to total volume of bottle

- **BPF binding buffer** and **WPF1 washing buffer** may form precipitates during storage which is not a quality failure. In this case, heat the buffer in the tightly closed bottle up to 45 °C and incubate until completely resolved.

Procedure

Work under sterile conditions at all steps!

1. Measure up to 20.0 g of milled grain into a glass beaker or tube (volume >200 ml) and add a stirrer; if available, a mash bath will fit best
2. Add 180 ml of LPT buffer (dilute before first use! ref. above); this step will disrupt/lyse the cell walls
3. Stir for 10 min at 65 °C (mash bath or thermoincubator)
4. In the meantime, prepare
    a) fresh reaction tube(s) (1 per sample is needed) as follows: Pipet 300 µl BPF binding buffer into a fresh reaction tube
    b) **Microspin column** (1 per sample is needed): place a spin column into an empty **2.0 ml** receiver tube (supplied with the kit)
5. Centrifuge an aliquot (8-10 ml) of the lysed sample from 3. at 3,000 g for 10 min
6. Take off the supernatant carefully and filter an aliquot of 3-5 ml of the supernanant through a single use filter 0,45 µm pore size (use a syringe and a membrane filter unit for water soluble samples), collect filtered volume (=cleared lysate) in a fresh tube
7. Add 300 µl of cleared lysate (from 6.) into one tube with BPF buffer (from 4.a), mix thoroughly by shaking or vortexing
8. Add 100 µl of i-propanol per tube
9. Mix again well by shaking or vortexing
10. Add total volume from 9. onto a spin filter column from 4.b
11. Centrifuge for 1 min at 6,000 x g and discard the flow-through - **keep the column and receiver tube! and re-use both for the following steps** 13.-21.!
12. Put microspin column back into previously used receiver tube
13. In the meantime, heat buffer EP to 65 °C ± 5 °C
14. Add 500 µl of washing buffer WPF1 onto the microspin column
15. Centrifuge at 8,000 g for 1 min
16. Discard flow-through, put column back into the previously used receiver tube
17. Add 500 µl of washing buffer WPF2 onto the spin column
18. Centrifuge at 8,000 g for 1 min
19. Discard flow-through, put column back into the previously used receiver tube
20. Optional: Repeat steps 16. to 18.
21. Centrifuge empty microspin column again (without addition of buffer) at 11,000 g for 2 min – take care that the column is dry afterwards; if not so, turn the spin column in the receiver tube through 180 degrees and repeat centrifugation. *This step ensures that no ethanol is carried over to the following DNA elution process; ethanol will inhibit elution.*

22. Put microspin column into a **fresh** 1.5 ml collection tube (included in the kit)
23. Add 100 µl of **pre-heated** (65 °C ± 5 °C) elution buffer EP
24. Incubate at room temperature for 5 min
25. Centrifuge at 11,000 g for 1 min
26. The flow-through is containing the DNA and may be used for further analyses
27. Optional: Repeat steps 23 to 26 for 2nd elution which may improve the yield rate
28. For short storage of the DNA, 4 °C are appropriate, for long term storage at –20 °C is strictly recommended
29. The isolated DNA is ready for further analyses, e.g. PCR applications.

Further analyses

For the analysis of *Fusarium* in grain, our product **TM Detection Kit S *Fusarium* sp. Screening** (item no. 2003-22) may be used.

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