INSTRACTION FOR USE
The YZZY-RNA MAGNET KIT

INTENDED USE

The YZZY-RNA MAGNET Kit is intended for isolation of total DNA/RNA from clinical material: smears, scrapings and mucousa of the urogenital, respiratory and digestive tracts, biological fluids (plasma, serum, saliva, detachable conjunctiva, cerebrospinal fluid, pleural, synovial and amniotic fluids). Isolated DNA/RNA is suitable for further molecular biological studies, including RT-PCR.

PERFORMANCE OF YZZY-RNA MAGNET KIT REAGENTS

The YZZY-RNA MAGNET Kit is a highly sensitive Kit that allows efficient isolation both RNA and DNA from clinical material. The sensitivity limit is 10 genome equivalents per extraction.
The YZZY-RNA MAGNET Kit allows to conduct both standard and high sensitivity studies with a sample volume of up to 100 µl.
The YZZY-RNA MAGNET Kit allows to purify RNA/DNA both in manual mode (use magnetic stand for single PCR tubes 1,5/2,0 ml or handheld Magnetic stand 96 for the separation of magnetic beads in 96-well format plates) and in automatic mode using open robotic stations.
Purification of RNA/DNA using the YZZY-RNA MAGNET Kit is simple and convenient and does not require additional reagents.
The YZZY-RNA MAGNET Kit set of reagents are designed to conduct studies of 100 samples with a volume of 100 µl (table 1).

Table 1. The components composition of YZZY-RNA MAGNET Kit

Name of component	Volume
1. Magnetic sorbent	1 tube – 1.6 ml
2. Sorbing solution	1 bottle – 45 ml
3. Wash solution 1	1 bottle – 50 ml
4. Wash solution 2	1 bottle – 50 ml
5. Elution solution	1 bottle – 15 ml

PRECAUTIONARY MEASURES

When working with YZZY-RNA MAGNET Kit's reagents, it is necessary to observe the safety measures established for the appropriate sphere of clinical laboratory PCR diagnostics.
While working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.

REQUIRED EQUIPMENT AND MATERIALS

- laminar box or PCR-box;
- Thermo–Shaker for Deep Well Plates with heating temperature up to 65 °C such as TS-DW (Biasan) if 96 Deep Well PCR Plate using / thermo-shaker, heated orbital incubator, Dry Block Thermostats with heating temperature up to 65 °C if Single PCR tube 1.5/2.0 ml using;
- Benchtop centrifuge with angle rotor 1.5/2.0 ml tubes (RCF ≥ 10,000 × g) if Single PCR tube 1.5/2.0 ml using or centrifuge with rotor for 96 Deep Well PCR Plate (RCF ≥ 3,000 × g);
- vortex;
- one channel and 8-chennel pipettes with variable volume (10 – 1000 µl);
- pipette tips (to avoid cross-contamination, we recommend pipet tips with aerosol barriers);
- 96 Deep Well PCR U-bottom Plates such as Eppendorf® 96/1000 µl, Sarstedt® Megablock 96/2200 µl, Axygen® 96/2200 µl or Single PCR tubes 1.5/2.0 ml;
- seals for 96-well PCR Plates if 96 Deep Well PCR Plates;
- handheld Magnetic stand 96 for the separation of magnetic beads in 96-well format plates such as ZR-96 MagStand (Zymo Research), Invitrogen™ Magnetic Stand-96 (Thermo Fisher Scientific) or handheld Magnetic stand for single PCR tubes 1.5/2.0 ml;
- aspirator with Trap Flask;
- refrigerator with freezing camera (temperature from -24 °C to +8 °C);
- disposable gloves;
- waist tank;
- workstation disinfectant Kit.

When working with RNA, it is necessary to use consumables that have a special marking "RNase-free", "DNase-free".

PREPARTION OF THE TEST SAMPLES

Collection, transportation and storage of the samples to be tested is carried out in accordance with applicable rules and instructions.

Samples of urine are requiring pre-processing in accordance with generally accepted methodological recommendations.

All other samples are thoroughly homogenized with a vortex immediately prior to DNA/RNA extraction; drops of material from the inside of the lid are removed by short-term centrifugation at minimum speeds.

RNA/DNA EXTRACTION FROM 100 µL SAMPLE USING MAGNETIC SEPARATION STAND

0. **If Sorbing solution and/or Wash solution 1 contains precipitate, it need to be dissolved by heating to 55-60°C with gentle agitation. Carefully re-suspend the Magnetic sorbent on the vortex for the period of 10 s before starting extraction procedure.**

I. Prepare the mixtures of **Sorbing solution, Magnetic sorbent** and **Internal extraction control** (in case of use) at the rate of **450 µl of Sorbing solution**, **16 µl of Magnetic sorbent** and **10 µl of Internal extraction control** (when using) for one isolation at container of the corresponding volume. Mix the resulting mixture on a vortex. **Table 2** shows the calculation of the components volumes according to

certain samples quantity. It is recommended to add the entire volume of the **Magnetic sorbent** and **Internal extraction control into** a bottle with a **Sorbing solution** if making one-time extraction of 100 samples.

Table 2. The Ratios of components.

The quantity of samples	Sorbing solution (ml)	Magnetic sorbent (ml)	Internal extraction control (ml)	The quantity of samples	Sorbing solution (ml)	Magnetic sorbent (ml)	Internal extraction control (ml)
1	0,45	0,016	10	**55**	24,75	0,88	550
5	2,25	0,08	50	**60**	27	0,96	600
10	4,5	0,16	100	**65**	29,25	1,04	650
15	6,75	0,24	150	**70**	31,5	1,12	700
20	9	0,32	200	**75**	33,75	1,2	750
25	11,25	0,4	250	**80**	36	1,28	800
30	13,5	0,48	300	**85**	38,25	1,36	850
35	15,75	0,56	350	**90**	40,5	1,44	900
40	18	0,64	400	**95**	42,75	1,52	950
45	20,25	0,72	450	**100**	45	1,6	1000
50	22,5	0,8	500				

II. Add **466 µl** or **476 µl** (if using internal control) of the **prepared mixture** (step I) into 1.5/2 ml PCR tube or 96 Deep Well PCR Plate.

III. Add **100 µl of the sample** in each PCR tube or well of 96 Deep Well PCR Plate (see page 1, PREPARTION OF THE TEST SAMPLES). Seal the lids.

IV. **A.** Mix tubes content by pulse-vortexing for **5-10 s** and incubate at **65°C for 5 min** with constant (recommended) or periodical mixing (pulse-vortexing for 2 times).

B. If 96 Deep Well PCR Plate using place PCR Plate into Thermo-Shaker's block, incubate at **65°C for 5 min shaking at 1400 rpm.**

V. Remove drops from the lids by short-term centrifugation at minimum speeds or centrifuge at **280 × g for 1 min**.

VI. Place PCR tubes/96 Deep Well PCR Plate on the magnetic separation stand for **30-60 s** period. Carefully remove supernatant avoiding touching the magnetic sorbent.

VII. Add **500 µl of Wash solution 1** and seal the lids.

VIII. Mix tubes content by pulse-vortexing for **5-10 s** if PCR tubes using / place PCR Plate into Thermo-Shaker's block, incubate at **ambient temperature for 10 s shaking at 1400 rpm.**

IX. Remove drops from the lids by short-term centrifugation at minimum speeds or centrifuge at **280 × g for 1 min**.

X. Transfer PCR tubes/96 Deep Well PCR Plate on a magnetic separation stand for **30-60 s**. Carefully remove supernatant avoiding touching the magnetic sorbent.

XI. Add **500 µl of Wash solution 2** and seal the lids.

XII. Mix tubes content by pulse-vortexing for **5-10 s** if PCR tubes using / place PCR Plate into Thermo-Shaker's block, incubate at **ambient temperature for 10 s shaking at 1400 rpm.**

XIII. Remove drops from the lids by short-term centrifugation at minimum speed or centrifuge at **280 × g for 1 min**.

XIV. Place PCR tubes/96 Deep Well PCR Plate on the magnetic separation stand for **30-60 s** period. Carefully remove supernatant without touching the magnetic sorbent.

At this stage, it is necessary to remove cautiously the residue of Wash Solution 2, because it can cause inhibition of PCR.

XV. Dry PCR tubes/96 Deep Well PCR Plate with open lids for **1 min**.

XVI. Add **100 µl of Elution solution** and mix tubes content by pulse-vortexing for **5-30 s** if PCR tubes using. Seal the lids.

XVII. **A.** If PCR tubes using incubate the tubes at **65°C for 5 min** with constant (recommend) or periodical mixing (pulse-vortexing for 5-10 s every 2 minutes). After the incubation end mix tubes by pulse-vortexing for **2 s**.

B. If 96 Deep Well PCR Plate using place PCR Plate into Thermo-Shaker's block, incubate at **65°C for 5 min shaking at 1400 rpm.**

XVIII. Remove drops from the lids by short-term centrifugation at minimum speed or centrifuge at **280 × g for 1 min**.

XIX. Place PCR tubes/96 Deep Well PCR Plate on the magnetic separation stand for **30-60 s**. Supernatant contains purified RNA and DNA ready for reverse transcription and PCR.

It is strongly recommended to transfer the supernatant to new PCR tubes (96-well PCR Plate) for long-term sample storage. Storage of samples is allowed for the period of 24 hours at the temperature of not higher than 4 °C or for 1 year at a temperature of not higher than -16 °C.

TRANSPORTATION AND STORAGE OF YZZY-RNA MAGNET KIT

Transportation period of The YZZY-RNA MAGNET Kit is 3 days at normal ambient temperature (but not more than 30 °C).
The YZZY-RNA MAGNET Kit may be transported by any type of transport in conditions ensuring it safety, in accordance with the rules for the goods carriage for this type of transport.
The YZZY-RNA MAGNET Kit is stored at the manufacturer's packaging at +2 - +8 °C temperature for the entire shelf life.
The shelf life of YZZY-RNA MAGNET Kit is 12 months from the date of manufacture.