Quick-Start Protocol August 2016

Heated off-Board Lysis Protocol: Purification of pathogen nucleic acids from fluid samples using the MagAttract® 96 *cador*® Pathogen Kit

The MagAttract 96 *cador* Pathogen Kit (384) (cat. no. 947457) can be stored at room temperature (15–25°C). For expiry date information, consult the label on the kit box.

Further information

- *MagAttract 96* cador *Pathogen Handbook*: **www.qiagen.com/handbooks**
- Technical assistance: toll-free 00800-22-44-6000, or **www.qiagen.com/contact**

Equipment and reagents to be supplied by user

- Adhesive tape or Tape Pads (cat. no. 19570)
- Thermoshaker suitable for S-blocks
- Optional: Centrifuge suitable for S-blocks

Important notes before starting

- Read the safety information in the *BioSprint® 96 User Manual* before use.
- Dissolve carrier RNA in Buffer AVE as indicated on the tube.
- Add isopropanol (100%) to Buffer ACB and ethanol (96–100%) to Buffers AW1 and AW2 before use. See the respective bottle labels for volumes.
- If using frozen samples, equilibrate to room temperature (15–25°C).
- If your sample volume is less than 200 µl, bring it to 200 µl with PBS or 0.9% NaCl.

- 96-Rod Covers are supplied as packs of 1 or 2, inserted into an S-Block. If using a pack of 2 covers, store the second cover in another S-Block or plate. Take care to not bend the covers.
- Heat a thermoshaker to 70°C for use in step 5.

Procedure

1. Pipet 20 µl Proteinase K into the bottom of an S-Block.
2. Add 200 µl fluid sample to the Proteinase K.

   Note: If your sample volume is less than 200 µl, bring it to 200 µl with PBS or 0.9% NaCl.
3. Add 100 µl Buffer VXL mixture to each sample in the S-Block.
4. Cover the S-Block with adhesive tape.
5. Incubate at 70°C, with constant agitation, for 10 min.
6. Optional: Briefly centrifuge the S-block to remove drops from the inside of the tape.
7. Remove the adhesive tape from the S-Block.
8. Prepare the Buffer ACB mixture (see Table 1) and mix thoroughly for 30s.

Table 1. Buffer ACB mixture preparation

	Number of samples*		
Reagent	1	48	96
Buffer ACB	400 µl	19.2 ml	38.4 ml
MagAttract Suspension G	25 µl	1.2 ml	2.4 ml
Carrier RNA (1 µg/µl)	1 µl	48 µl	96 µl

* Prepared volume is 107% of required volume, to compensate for pipetting errors and possible evaporation.

9. Add 400 µl Buffer ACB mixture to each sample in the S-Block.
10. Prepare 4 additional S-Blocks (slots 2–6) and one 96-Well Microplate MP, according to Table 2.

Table 2. BioSprint 96 worktable setup and reagent volumes

Slot	Loading message	Format	Item to add	Volume per well (µl)
6	Load Rod Cover	S-Block	Large 96-Rod Cover	—
5	Load Elution	96-Well Microplate MP	Buffer AVE	100
4	Load Wash 3	S-Block	Ethanol (96–100%)	750
3	Load Wash 2	S-Block	Buffer AW2	700
2	Load Wash 1	S-Block	Buffer AW1	700
1	Load Lysate	S-Block	Lysate*	720

* Includes 20 µl Proteinase K, 200 µl sample, 100 µl Buffer VXL mixture and 400 µl Buffer ACB mixture.

11. Switch on the BioSprint 96 at the power switch.
12. Slide open the front door of the protective cover.
13. Select the ''BS96 cador v2'' protocol using the ▲ and ▼ keys.
14. Press "Start" and follow the messages for loading the worktable, as shown in Table 2.

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