Quick-Start Protocol October 2016

MagAttract® Mastitis Kit

The MagAttract Mastitis Kit can be stored dry at room temperature (15–25°C) for up to 1 year without showing any reduction in performance.

Further information

- *MagAttract Mastitis Kit Handbook*: **www.qiagen.com/handbooks**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: toll-free 00800-22-44-6000 or **www.qiagen.com/contact**

Notes before starting

- Check that Buffer MVL, Buffer AW1 and Buffer AW2 have been prepared according to the instructions in the *MagAttract Mastitis Kit Handbook*.
- The 96-rod covers are supplied either as packets of 2, or as packets of 1 inserted into an S-Block. If using a new packet of 2, store the second 96-rod cover on another S-block or plate. Care should be taken to not bend the 96-rod covers.
- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge/Centrifuge 4-16S.
- Use of a multichannel pipet is recommended.
- As a starting material, 400 µl of fresh, frozen or stabilized milk should be used.
- Thaw and equilibrate up to 96 samples at room temperature (15–25°C).
- Prepare the ML and MVL mixture according to Table 1 and Table 2. Before adding MagAttract Suspension G, ensure that it is fully resuspended. Vortex for 3 minutes before using for the first time, and for 1 minute before subsequent use.

Table 1. Preparation of MVL mixture

	Number of samples*			
Reagent	**1**	**8**	**48**	**96**
Buffer MVL	0.5 ml	4 ml	24 ml	48 ml
MagAttract Suspension G	25 µl	200 µl	1200 µl	2400 µl

* The volume prepared is 105% of the required volume to compensate for pipetting error and possible evaporation.

Table 2. Buffer ML mixture preparation

	Number of samples†			
Reagent	**1**	**8**	**48**	**96**
Buffer ML	80 µl	640 µl	3840 µl	7680 µl
Reagent DX	1 µl	8 µl	48 µl	96 µl

† Calculate 1–2 extra reactions to ensure sufficient volume.

Protocol

1. Label 6 x S-Blocks.
2. Prepare Buffer MVL mixture (see Table 1) and mix thoroughly by flicking the tube.
3. Add 500 µl Buffer MVL mixture to each well in the S-Block.
4. Prepare 5 additional S-Blocks (slots 2–6) according to Table 3.

Table 3. 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 ATE	100
4	Load Wash 3	S-Block	Ethanol (96–100%)	1000
3	Load Wash 2	S-Block	Buffer AW2	1000
2	Load Wash 1	S-Block	Buffer AW1	1000
1	Load Lysate	S-Block	Lysate*	920

* Includes 420 µl lysate and 500 µl Buffer MVL mixture.

5. Mix the sample thoroughly by vortexing.

6. Open Pathogen Lysis Microtubes S and discard caps.
7. Add 80 µl Buffer ML mixture (see Table 2) to each tube.
8. Pipet 400 µl sample into the Pathogen Lysis Microtubes S by touching the insides of the tubes without wetting the rims.
9. Cover the rack with new caps for collection microtubes (provided).
10. Homogenize the sample until the sample is thoroughly homogenized.

    Disruption and homogenization using the TissueLyser II

    10a. Place the Pathogen Lysis Microtubes S in the TissueLyser Adapter Set 2 x 96.

    10b. Operate the TissueLyser II for 8 min at 30 Hz.

    10c. Rearrange the tubes so that the outermost tubes are innermost, and the innermost tubes are outermost.

    10d. Operate the TissueLyser II for another 8 min at 30 Hz.

11. Centrifuge briefly to remove drops from the inside of the tube lid.
12. Carefully apply all of the lysate (approximately 420 µl) from step 11 to the first S-block (Lysate).
13. Switch the BioSprint® 96 on at the power switch.
14. Slide the front door of the protective cover open.
15. Select the protocol ''BS96 mastitis'' using the ▲ and ▼ keys.
16. Press "Start" and follow the messages for loading the worktable as shown in Table 3.
17. The LCD displays a message asking you to load slot 6 of the worktable with the 96-rod cover (see Table 3 above). After loading slot 6, press "Start". The worktable rotates and a new message appears, asking you to load slot 5 with the elution plate. Load slot 5 and press "Start" again. Continue this process of pressing "Start" and loading a particular slot until all slots are loaded.

    Each slot is labeled with a number. Load each plate or block so that well A1 is aligned with the slot label (i.e., well A1 faces inward).

18. Check that the protective cover is correctly installed: it should fit exactly into the body of the BioSprint 96. Slide the door shut to protect samples from contamination.
19. Press “Start” to start sample processing.
20. After the samples are processed, remove the plates and blocks as instructed by the display of the BioSprint 96. Press “Start” after removing each plate or block. The first item to be removed contains the purified samples.
21. Press “Stop” after all plates and blocks are removed.
22. Discard the used blocks and 96-rod cover according to your local safety regulations.
23. Switch off the BioSprint 96 at the power switch.
24. Wipe the worktable and adjacent surfaces using a soft cloth or tissue moistened with distilled water or detergent solution. If infectious material is spilt on the worktable, clean using 70% ethanol or other disinfectant.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.