Phadebact®
Coating Kit
For research use only

Directions for Use

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INTENDED USE
To make your own co-agglutination reagents.

PRINCIPLE OF THE PROCEDURE
The first to use fixed S. aureus cells for diagnostic purposes was Professor G. Kronvall. He used the cells to create a typing reagent in a visual agglutination test for rapid serotyping of pneumococci (ref). Later, many applications using this technique have been developed including typing of E. coli (ref), Haemophilus influenzae (ref), Salmonella (ref), Neisseria meningitidis (ref), Klebsiella pneumoniae (ref), Shigella (ref), streptococci (ref), meningococci (ref). Phadebact® Coating Kit consists of formalinized and heat killed S. aureus cells. To make your own co-agglutination reagent staphylococcal cells are first coated with specific antisera (or monoclonal antibodies) to the antigen to be detected. The Fc-part of the antibodies will bind to the surface-protein A on the staphylococci, orienting the Fab-part outwards. A positive reaction, when antigens bind to the antibodies on the staphylococcal cells, results in an agglutination visible to the eye.

To facilitate the interpretation of the result, the reagent is dyed blue with methylene blue.

Since the amount of antibodies needed for a specific test varies, different concentrations of serum are to be coated. Too small amount of antibodies gives weak or no reaction. Excess amount of antibodies can give unwanted cross-reactivity and waste of precious antibodies. Our recommendations are shown in the instructions below.

REAGENTS
Each Phadebact® Coating Kit package contains reagents sufficient for 50 determinations. The reagents are coloured blue (Methylene blue) to facilitate interpretation of results.

Reactive ingredients
• S. aureus cells dyed with methylene blue 2 vials

Other components
• 2 empty vials for prepared reagents
• Labels
• Droppers
• Disposable slides
• Directions for Use

Recepie
PBS-azide
K$_2$HPO$_4$ 5.60 g
KH$_2$PO$_4$ 0.74 g
NaCl 7.01 g
NaN$_3$ 1.00 g
Purified water 1000 mL

Precaution
For in vitro diagnostic use.

Warning! The reagents contain sodium azide (NaN$_3$) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC.

Shelf life and storage
The expiry date is stated on the outer label and on the vial labels. It is recommended that the kit be stored at 2-8°C in an upright position. Reagents must be protected from freezing.

Preparation of reagents
Two different concentrations of antisera should preferably be tested. If monoclonal antibodies are used, 0.1 and 0.02 mg antibodies to 1 mL 10% staphylococcal suspension is suggested amount to be added. If total antisera is used, 0.1 and 0.02 mL antibodies should be coated.

1. Shake the vial vigorously to resuspend the cells totally.
2. Transfer the S. aureus cells from the vial to a 10 mL centrifugation tube.
3. Centrifuge 20 min, 3000 rpm.
4. Discard the supernatant.
5. Take the recommended amount of antisera/antibodies and dilute to a final volume of 4.9 mL with PBS-azide. Add the mixture.
6. Resuspend homogeneously and incubate one hour “end over end” at room temperature.
7. Centrifuge the coagglutinated staphylococci 20 min, 3000 rpm.
8. Discard the supernatant and resuspend in 4.9 mL of PBS-azide.
9. Centrifuge the staphylococci 20 min, 3000 rpm.
10. Discard the supernatant and resuspend the Phadebact® cells in 4.9 mL of PBS-azide.

Note! We do not recommend that each vial is divided in more than two coatings as the pellet will be small and difficult to work with.
**PROCEDURE**

*Note! Let the reagents attain room temperature and suspend reagents thoroughly by shaking or vortexing before use.

**Put one drop of your reagent on the marked places on the slide. Fig. 1.**

**Put one drop of bacterial suspension beside the reagent. Fig. 2.**

**Mix each pair of drops with a disposable loop. Use a fresh loop for each reagent. Fig. 3.**

**Rock the slide and read the result within 1 minute. Fig. 4.**

**Stability of the final reaction mixture**

The co-agglutination is a stable and good test. However, the methodology dictates that the result is read within 1 minute while the mixture still is wet (observe the risk of dryed reagents which can be misinterpreted as a reaction).

**Negative control reagent**

There are two ways to make a negative control. Either you take normal rabbit or mouse sera and follow the coat instructions or use other typing reagents as negative control. A reaction in both specific and negative control reagent indicates unspecific cross-reactivity.

**WARRANTY**

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by MKL Diagnostics AB may affect the results, in which event MKL Diagnostics AB disclaims all warranties, expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. MKL Diagnostics AB and its authorized distributors, in such event, shall not be liable for any damages, whether direct, indirect or consequential.

**Bibliography:**

21. Data on file, MKL Diagnostics AB.
PRODUCTS

**Phadebact® COA System**
- Phadebact® Streptococcus Tests
- Phadebact® Streptococcus Respiratory Test
- Phadebact® Strep A Test
- Phadebact® Strep B Test
- Phadebact® Strep D Tests
- Phadebact® Strep F Test
- Phadebact® Strep Positive Controls
- Phadebact® Pneumococcus Test
- Phadebact® Haemophilus Test
- Phadebact® GC Positive Controls
- Phadebact® CSF Test
- Phadebact® CSF Positive Controls
- Phadebact® Extraction Solutions
- Phadebact® Monoclonal GC Test
- Phadebact® ETEC-LT Test
- Phadebact® Salmonella Test
- Phadebact® Staph Aureus Test

**Near Patient Testing**
- Phadirect® Strep A
- Phadirect® Rapid CRP Test