



BD GeneOhm™ MRSA ACP Lysis Kit



IVD

REF 441638 100 Tests

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ENGLISH

Intended Use

The BD GeneOhm™ MRSA ACP Lysis Kit provides a simplified method for lysing *Staphylococcus aureus* cells from nasal swab specimens prior to analysis with the BD GeneOhm™ MRSA ACP Assay.

Summary and Explanation of the Procedure

The BD GeneOhm™ MRSA ACP Lysis Kit incorporates enzymatic Achromopeptidase (ACP) lysis technology for sample preparation of nasal swab specimens used with the BD GeneOhm™ MRSA ACP Assay. Lysis Reagent is reconstituted with Lysis Diluent and aliquoted into single use lysis tubes. A nasal swab specimen is collected and transported to the laboratory using the recommended swab transport device (refer to "Materials Required but Not Provided"). The nasal swab is broken into a Sample Buffer tube. An aliquot of the liquid sample suspension is then transferred into the tube containing the lysis reagent and incubated to break the bacterial cell wall and release the cell's genetic material. An aliquot of the lysed sample is then processed with the BD GeneOhm™ MRSA ACP Assay.

Reagents

BD GeneOhm™ MRSA ACP Lysis Kit	Quantity
<i>Lysis Diluent</i>	1 x 3.7 mL
Tris-EDTA buffer for enzyme reconstitution	
<i>Lysis Reagent</i>	1 bottle
Dried lysis bulk ACP enzyme (15.1 KU)	
<i>Sample Buffer</i>	162 x 600 µL
Tris-EDTA buffer for sample and negative control preparation, and positive control DNA reconstitution	

Precautions

- For *in vitro* diagnostic use.
- Do not use the kit if the outer carton safety seal is broken.
- Do not use reagents if the protective pouches are open or torn upon arrival.
- Close Sample Buffer protective pouches promptly with the zip seal after each use.
- Do not use Lysis Reagent if desiccant is not present or is broken inside the pouch.
- Do not pool reagents or interchange reagent lots.
- Do not use the reagents after their expiration dates.
- Avoid microbial and deoxyribonuclease (DNase) contamination of reagents when removing aliquots from tubes. The use of sterile DNase-free disposable filter-blocked or positive displacement pipettor tips is recommended.
- Although the main technical operation is pipetting, good laboratory technique is essential to the proper performance of this procedure. Due to the high analytical sensitivity of the BD GeneOhm™ MRSA ACP Assay, extreme care should be taken to preserve the purity of all reagents, especially in cases where multiple aliquots are removed from a tube.
- Use a pipettor tip with a diameter small enough to reach the liquid at the bottom of each specimen or reagent tube.
- Performing the lysis steps outside recommended time ranges may affect DNA amplification and produce invalid results. Lysis not performed within specified time ranges should be repeated.
- Additional controls may be tested according to guidelines or requirements of local, state, provincial and/or federal regulations or accrediting organizations.
- In cases where open-tube PCR tests are conducted in the same general area of the laboratory, separated and segregated work areas should be used for specimen preparation and amplification/detection activities. Supplies and equipment should be dedicated to each area and

should not be moved from one area to another. Gloves must always be worn and must be changed before moving from one area to another.

- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in *Biosafety in Microbiological and Biomedical Laboratories*¹ and in the-CLSI Document M29².
- Wear protective clothing and disposable gloves while handling kit reagents. Wash hands thoroughly after performing the test.
- Do not pipet by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state and local regulations.

Materials Provided

- Lysis Diluent
- Lysis Reagent
- Sample Buffer tubes
- Screw caps
- Septum caps
- Storage tubes

Storage, Handling and Stability

Collected Specimens

Specimens should be kept between 2°C and 25°C during transport. Protect against freezing or exposure to excessive heat. Specimens can be stored up to 48 hours at 15-25°C or 5 days at 2-8°C before testing.

Sample Buffer Containing the Eluted Sample

The eluted sample in the Sample Buffer tube is stable up to 72 hours at 2-8°C (e.g. for repeat testing).

Specimen and Control Lysates

Specimens and control lysates are stable up to 4 hours at 2-8°C with either septum or screw cap, or 8 days at -20°C with a screw cap.

Reagents

Note: Storage conditions must follow the specifications written on each pouch.

Sample Buffer (blue cap) Storage Conditions		
Sealed pouch	Temperature	2-25°C
	Stability	Expiration date
Opened pouch	Temperature ¹	2-25°C
	Stability ²	1 month

¹ Although these reagents can be stored at room temperature, they should be kept with their accompanying lysis reagent of the same lot at 2-8°C.

² Provided the pouch is properly closed with the zip seal after each use.

Lysis Reagent Storage Conditions			
Lysis Reagent (Unreconstituted in Bottle)	15.1 KU	Temperature	2-25°C
		Stability	Expiration
Reconstituted and aliquoted in the lysis tube closed with a septum cap	20 µL	Temperature	2-8°C
		Stability	1 week
Reconstituted and aliquoted in the lysis tube closed with a screw-cap	20 µL	Temperature	2-8°C
		Stability	1 month

Materials Required but Not Provided

- BBL™ CultureSwab™ Liquid Stuart single or double swab (Becton Dickinson catalog no. 220099 or 220109), Copan (Venturi) Transystem™ Liquid Stuart single or double swab (Copan, catalog no. 141C or 139C), or
- BBL™ CultureSwab™ Liquid Amies single or double swab (Becton Dickinson catalog no. 220093 or 220105), Copan (Venturi) Transystem™ Liquid Amies single or double swab (Copan, catalog no. 140C or 138C), or
- BBL™ CultureSwab™ Plus Amies Gel without Charcoal single or double swab (Becton Dickinson catalog no. 220116 or 220117), Copan (Venturi) Transystem™ Amies Agar Gel without Charcoal single or double swab (Copan, catalog no. 108C or 134C)
- **BD GeneOhm™ MRSA ACP Assay** (BD catalog no. 441637 or 441639)
- **Vortex** Genie 2 (VWR catalog no. 58815-234) with 1.5 mL microtube holder or equivalent; for processing multiple samples, adaptor designed for multiple tubes can be used (VWR catalog no. 58816-146) **OR** VWR Signature Multi-Tube **Vortexer** (VWR 58516-115).
- **Dry heating block** specific for 1.5 mL tubes at 37+/-2 °C
- **Dry heating block** specific for 1.5 mL tubes at 99+/-2 °C
- Ice or **cooling block** for 1.5 mL tubes
- **Calibrated Micropipettors** (accurate range between 10-100 µL and 100-1000 µL)
- **Sterile DNase-free** filter-blocked or positive displacement profiled **micropipettor tips** (10-100 µL and 100-1000 µL)
- Gram staining reagent (optional)
- BBL™ CHROMagar™ Staph aureus (BD Diagnostic Systems catalog no. 214982), BBL™ CHROMagar™ MRSA (BD Diagnostic Systems catalog no. 215084), Mannitol Salt Agar (MSA) (BD Diagnostic Systems catalog no. 221173 or 221271 or equivalent media) (optional)
- **TSB** (trypticase soy broth) supplemented with 6.5% NaCl (BD Diagnostic Systems catalog no. 221351) (optional)
- 5% sheep blood agar plate (e.g. BBL™ Trypticase Soy Agar (TSA II) with 5% Sheep Blood, BD Diagnostic Systems catalog no. 221239 or 221261) (optional)
- Disposable **gloves**, powderless
- **Scissors** (optional)
- **Gauze**
- Stopwatch or **timer**

Instructions for Use

Bulk Lysis Reagent Preparation and Storage

1. Pour the entire contents of the Lysis Diluent tube into the Lysis Reagent bottle.
2. Recap bottle tightly and vortex at high speed for 30 seconds.
3. Allow the reconstituted bulk Lysis Reagent to stand at room temperature for 20 minutes.
4. Vortex the Lysis Reagent bottle at high speed for 30 seconds.

Note: Once reconstituted, the entire contents of the Bulk Lysis Reagent bottle must be aliquoted into individual single use lysis tubes.

5. Using a repeat pipettor, dispense 20 µL of reconstituted Lysis Reagent into the single use lysis tubes. (1 bottle of bulk Lysis Reagent is sufficient for up to 160 reactions)
6. Close tubes with a septum cap or a screw cap and label with the appropriate expiration date.
7. Store Lysis tubes between 2-8°C.

Note: Lysis Reagent stored with a septum cap is stable for up to 7 days, and up to 30 days when stored with a screw cap (Refer to "Storage, Handling and Stability - Reagents" section).

Specimen Collection

Using a recommended swab transport device (refer to Material Required but Not Provided), nasal specimens should be collected according to hospital standard operating procedures and the following:

1. Moisten the swab with sterile physiological saline or use dry.
2. Carefully insert the swab into the patient's nostril (a swab tip should be inserted up to 2.5 cm (1 inch) from the edge of the nares).
3. Roll the swab along the mucosa inside the nostril 5 times.
4. Insert the same swab into the second nostril and repeat steps 2 and 3.
5. Replace the swab in its transport tube.
6. Label the transport tube.
7. Ship the swab to the laboratory according to hospital standard operating procedures (Refer to "Storage, Handling and Stability – Collected Specimens" section).

Specimen Preparation

Note: One (1) Sample Buffer tube (**blue cap**) is required for each specimen to be processed. Two (2) additional Sample Buffer tubes (**blue cap**) per run are needed for specimen processing controls (SPC), if necessary (refer to the "Quality Control" section below for details on preparation). Remove the required number of tubes from their protective pouch then **remove the excess air, and close the pouch promptly with the zip seal.** Ensure the protective pouch is properly sealed after closing.

Important: For culturing clinical specimens, refer to "Culturing of Clinical Specimens" section below.

1. Label each Sample Buffer (**blue cap**) tube with the appropriate identification.
2. Remove the swab from the sample transport tube and place the swab in the corresponding Sample Buffer tube (**blue cap**).
3. Break the swab stem and close the tube tightly with a screw cap.

Hold the swab by the stem near the rim of the tube (use gauze to minimize risk of contamination). Lift the swab approximately one (1) cm from the bottom (near the liquid level) and bend the stem against the edge of the tube to break it. Alternative method: use clean scissors to cut the stem.

4. Vortex the Sample Buffer tubes at high speed for 60 seconds.

For processing multiple samples, an adapter designed for multiple tubes may be used.

Specimen Lysis

Note: One (1) Sample Buffer tube (**blue cap**) is required for the reconstitution of the Control DNA (Positive Control) and for use as the Negative Control in each run. **One (1)** lysis tube containing 20 µL of aliquoted lysis reagent is required for **every specimen.** Two (2) additional lysis tubes per run are required for PCR controls. **One (1) Positive and one (1) Negative PCR Control must be included in each BD GeneOhm™ MRSA ACP Assay. One Control DNA tube (red strip label)**

from the **BD GeneOhm™ MRSA ACP Assay** is required per run. Remove the required number of tubes from their protective pouch, **remove the excess air and close the pouch promptly with the zip seal**. Ensure the protective pouch is properly sealed after closing.

1. Label each Lysis tube (20µL aliquot) with the appropriate identification.

2. Add 225 µL of Sample Buffer (blue cap) to the Control DNA tube.

Ensure that the Control DNA pellet is at the bottom of the tube. Insert the micropipettor tip through the septum of the Control DNA cap. Do not insert the tip too deeply into the cap. Dispense Sample Buffer into the tube. Save remaining Sample Buffer for the negative control.

3. Vortex the reconstituted Control DNA tube for 5-10 seconds.

Place the tube on ice or on a cooling block designed for 1.5 mL tubes until ready for processing.

4. Using a micropipettor with a new disposable pipette tip for each sample and control, add 90 µL of the eluted sample suspension directly into the appropriately labeled lysis tube through the septum or uncapped tube.

5. Add 90µL of Positive (Reconstituted DNA) control into a lysis tube.

Carefully remove the septum cap from the Positive Control tube before pipetting the Positive Control into the lysis tube. After pipetting, reseal the Positive Control tube with the original Positive Control septum cap. Retain Positive Control tube for use in repeat testing, if required.

6. Add 90µL of Negative (Sample Buffer) control into a lysis tube.

Sample Buffer tubes (specimen, controls, and SPCs, if available) can be stored at 2-8°C for up to 72 hours for follow up testing.

7. Incubate the Lysis tubes containing specimens and controls in a 37+/-2°C heating block for 20 minutes (lysis step).

Note: It is important to incubate the tubes within the specified temperature range.

8. Transfer Lysis tubes to a 99+/-2°C heating block and incubate for 5 minutes (inactivation step).

Note: It is important to incubate the tubes within the specified temperature range.

9. Transfer the Lysis tubes to a cooling block (2-8°C) and let stand for 10 minutes.

If lysates are not used within 4 hours, replace septum cap (if used) with screw cap and store tubes at -20°C.

10. Refer to the BD GeneOhm™ MRSA ACP Assay package insert to continue testing.

Quality Control

Assay (Positive and Negative) Controls

Quality control procedures are designed to monitor BD GeneOhm™ MRSA ACP Assay performance. The Positive Control is intended to monitor for substantial reagent failure. The Negative Control is used to detect reagent or environmental contamination (or carry-over) by either MRSA DNA or MRSA amplicons. One Positive and one Negative Control must be included in each assay run. Prepare controls according to instructions provided in the "Specimen Lysis" section of this document.

Specimen Processing (Positive and Negative) Controls

Specimen Processing Controls (SPCs) are recommended in order to provide assurance that significant cross contamination does not occur and to monitor for substantial reagent or methodology failure during the assay process. Additional control strains may be tested as SPCs according to guidelines or requirements of local, state and/or federal regulations or accreditation organizations. A reference MRSA strain (e.g. ATCC 43300) or a well characterized MRSA clinical isolate may be used as a positive SPC; MRSA MREJ type iii and vii strains, if available, may be used as additional positive SPC, to monitor assay probes and primers not directly controlled in the assay. A strain of methicillin susceptible *Staphylococcus aureus* (e.g. ATCC 25923) or any other non-*aureus* *Staphylococci* (e.g. *Staphylococcus epidermidis* ATCC 14990) may be used as a negative SPC.

SPCs are prepared as follows: Incubate colonies on 5% sheep blood agar for 18 to 24 hours. Resuspend isolated colonies in saline to a turbidity of 0.5 McFarland. Dilute with saline to obtain a suspension of ~10⁶ CFU/mL. Dip the recommended swab (refer to "Materials Required but Not Provided" section) into the bacterial suspension, press out the excess fluid and place in the properly identified Sample Buffer tube. Process and test as a specimen, along with appropriate PCR controls. Additional information can be found in the "Specimen Preparation" section above and in the BD GeneOhm™ MRSA ACP Assay package insert.

For general QC guidance, the user may wish to refer to CLSI MM3³ and C24.⁴

Culturing of Clinical Specimens

In order to perform antimicrobial susceptibility testing or epidemiological typing, clinical specimens may be cultured from the collection device (swab) prior to performing the sample preparation procedure (using the Streak-Plate method) or after the Specimen Preparation procedure (using the Enrichment Broth method). Swabs may be stored at 2-8°C in closed Sample Buffer tubes for up to 24 hours before culturing.

Streak-Plate Method

This culture method may be performed with specimen swabs prior to the specimen lysis procedure..

1. **Remove the collection device (swab) from its transport tube.**
2. **Inoculate an appropriate solid medium (e.g. a Mannitol salt agar (MSA) or a BBL™ CHROMagar™ MRSA plate) by streaking onto the first quadrant of the plate.**
3. **Return the swab to its transport tube or break it in a Sample Buffer tube (blue cap) of the BD GeneOhm™ MRSA ACP Lysis Kit and continue according to instructions in the “Specimen Preparation” section.**
4. **Using a sterile loop or needle, streak the inoculum onto the remaining quadrants.**
5. **Incubate the plate for 24-48 hours at 35 ± 2 °C.**
6. **Identify and confirm *S. aureus* colonies and test for methicillin resistance according to the plate manufacturer’s Instructions for Use.**

Enrichment Broth

This culture method may be performed with remaining swab in tubes after removal of eluted cell suspension for the specimen lysis step. Swabs may be stored at 2-8°C in closed Sample Buffer tubes for up to 24 hours before culturing.

1. **Transfer the remaining cell suspension to a microtube for future use, if necessary.**
2. **Add 1.0 mL of enrichment broth to Sample Buffer tube containing the swab.**
TSB (Trypticase Soy Broth) supplemented with 6.5% NaCl is recommended.
3. **Vortex inoculated enrichment broth for 2-5 seconds.**
4. **Incubate for 18-24 hours at 35 ± 2 °C. If no growth is visible, incubate for an additional 24 hours.**
5. **Subculture to an appropriate solid medium and incubate for 24-48 hours at 35 ± 2 °C (e.g. 5% sheep blood agar, MSA or BBL™CHROMagar™ MRSA plate).**
6. **Identify and confirm *S. aureus* colonies and test for methicillin resistance according to the plate manufacturer’s Instructions for Use.**

Limitations of the Procedure

- This product is intended for use only with the BD GeneOhm™ MRSA ACP Assay.
- This product is intended for use with nasal swab specimens collected in specimen collection and transport systems listed in the “Materials Required but Not Provided” section. Performance with specimen collection and transport systems other than those listed has not been evaluated.

Lysis Efficiency

Lysis efficiency was evaluated for each collection device type listed in the “Materials Required but Not Provided” section. Swab collection devices were inoculated with three (3) MRSA strains of known concentrations. An aliquot of eluted swab suspension was removed and processed according to the Sample Lysis instructions. Lysates were inoculated onto blood agar plates, incubated for 18-24 hours and CFUs were counted. Colony counts ranged from 0 to 1900 CFU/plate. A summary of CFU counts obtained from all collection devices used for each of the three (3) strains is presented in the table below. The lowest and highest lysis efficiencies observed were 99.7% and 100%, respectively.

MRSA Strain ¹	Collection Device Type (n = 18)	Initial CFU Count (lower bound)	Initial CFU Count (upper bound)	Final CFU Count After Lysis (lower bound)	Final CFU Count After Lysis (upper bound)	Lowest Lysis Efficiency ³ (%)	Highest Lysis Efficiency ⁴ (%)
A	Liquid Amies	7.26 x 10 ⁵	1.02 x 10 ⁶	0	700	99.9	100
	Gel Amies			0	500	99.9	100
	Liquid Stuart			0	800	99.9	100
B	Liquid Amies	5.47 x 10 ⁵	7.92 x 10 ⁵	5	1900	99.7	100
	Gel Amies			0	660	99.9	100
	Liquid Stuart			17	600	99.9	100
C	Liquid Amies ²	9.25 x 10 ⁵	1.19 x 10 ⁶	23	400	100	100
	Gel Amies			0	351	100	100
	Liquid Stuart ²			22	600	99.9	100

¹ A (ATCC 43300, MREJ Type ii), B (MREJ Type vii) and C (MREJ Type ii, PFGE USA 300), previously characterized MRSA strains

² In two cases, confluent growth was observed on one (1) of the plates (n = 17 instead of 18); therefore colony counts could not be performed.










³ The highest lysis efficiency was calculated as: [(the highest initial CFU count observed before lysis - the lowest count after lysis)/the highest initial CFU count observed before lysis] x 100.

⁴ The lowest lysis efficiency was calculated as: [(the lowest initial CFU count observed before lysis - the highest count after lysis)/the lowest initial CFU count observed before lysis] x 100.

References

- Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories. Richmond JY and McKinney RW (eds) (1993). HHS Publication number (CDC) 93-8395.
- Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids and Tissue; Approved Guideline, Document M29 (Refer to the latest edition).
- Clinical and Laboratory Standards Institute. Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline, Document MM3 (Refer to the latest edition).
- Clinical and Laboratory Standards Institute. Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline, Document C24 (Refer to the latest edition).

Index of Symbols

SYMBOL	MEANING
	Manufacturer
REF	Catalog number
	In Vitro Diagnostic Use
	Use by
	Contains sufficient for "n" tests
	Batch code
	Temperature limitation
	Protect from light and moisture
	Reseal pouches after use
	Consult instructions for use



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BENEX Limited, Bay K 1a/d,
Shannon Industrial, Estate,
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Ireland



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