INSTRUCTIONS FOR USE

SPUTAFLUID

1 - INTENDED USE

In vitro diagnostic device. Sputafluid is a sputum liquefying agent used to digest and thin out the sputum, thus enhancing the isolation of organisms responsible for chronic lung disease.

2 - COMPOSITION – VIAL CONTENT

Dithiothreitol in phosphate buffer 100 mg

Dithiothreitol (DTT) and phosphate buffer in precise amounts are lyophilised and supplied in individually labelled vials. Each vial is sufficient to obtain 100 mL of final product.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The diagnosis and management of chronic lung disease has improved with the advent of sputum fluidising agents. In the past, iodides and sodium salts have been used to help thin the dense bronchopulmonary secretions commonly associated with this disease, but with limited success.[1] A significant contribution was made by Sheffner in 1963, when he demonstrated that the reactive sulphydryl groups of n-acetylcysteine had mucolytic activity.[2] Later, Cleland demonstrated that the sulphydryl compound dithiothreitol (DTT) is a superior reagent for the specific and total reduction of disulphide bonds of mucoproteins.[3] DTT, as a fluidifying agent, is routinely used in sputum digestion prior to preparation of smears and cultures, as it does not affect the morphology, growth of pathogens in sputum.[4]

4 - METHOD OF PREPARATION

Reconstitute the contents of one vial of Sputafluid with 10 mL of sterile purified water using asepyic precautions. After closing the vial, shake gently for complete solubilisation of the lyophilised product. The resulting solution should be clear and free of particulate matter. Add the contents of the vial to a volume of sterile distilled water to a final volume of 100 mL.

5 - PHYSICAL CHARACTERISTICS

Appearance of lyophilised product	compact, white pellet
Appearance of reconstituted product	clear, colourless solution, without suspension
pH at 20-25°C	7.0 ± 0.1

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Sputafluid	Freeze-dried sputum liquefying agent	224001	4 vials, each for 100 mL of liquefying solution

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile microbiology loops and swabs, centrifuge tubes, thermostat and laboratory equipment, NaOH 1%, culture media.

8 - SPECIMENS

Sputum expectorated from the lower respiratory tract (bronchi, lungs)

9 - TEST PROCEDURE

1. In a centrifuge tube, add to the sputum sample an equal volume of diluted Sputafluid prepared as described above.
2. Vortex the sputum for 30 seconds.
3. Leave the mixture at room temperature for 15 minutes.

Prolonged exposure time of the solution at room temperature does not inhibit the growth of flora present in the sample.

For predominant organisms

1. Centrifuge the mixture for 5 minutes at 1500 rpm to sediment the cells.
2. Discard the supernatant and resuspend the sediment in a small amount of Sputafluid. The amount of diluent used depends on the volume of the sediment and the final concentration to be obtained. For colony counting a dilution of 1:100 with an inoculum of 0.01mL is recommended. For more accurate bacterial counts, serial dilutions are required.

For acid-fast bacilli

1. Decontaminate the specimen by suspending the sediment in 5-10 mL of 1% NaOH (thorough shaking in the first minute is required).
2. Centrifuge the suspension for 15 minutes at 3000 rpm and discard the supernatant.
3. Wash the sediment twice with 10 mL of Sputafluid.
4. After the last centrifugation, resuspend the sediment in 0.5 mL of Sputafluid.
5. Culture on specific media to isolate acid-fast bacilli.

10 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory.

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all batches of Sputafluid is tested for the absence of growth inhibitory activity of the following microorganisms: *H.influenzae* ATCC 10211, *S.pneumoniae* ATCC 6305.

12 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is classified as dangerous according to the current European legislation. Consult the Safety Data Sheet before use.
- During and after use, handle all materials in a manner consistent with Good Laboratory Practice and always bear in mind that the material under test must be considered a potential biohazard if mishandled.

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- The laboratory area must be controlled to avoid contaminants such as the strips, the culture media or the microbial strains.
- Sterilize all biohazard waste before disposal. Dispose the unused reagent and the plates inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

13 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

14 – REFERENCES

1. Hirsh SR, Zastrow JE, Kory, RC. Sputum liquefying agents: a comparative “in vitro evaluation”. J Lab & Clin Med 1969; 74: 346-352.
2. Shah RJ, Dye, WE. Use of dithiothreitol to replace n-acetyl-l-cysteine for routine sputum digestion-decontamination for the culture of mycobacteria Am Rev Respir Dis 1966; 94: 454
3. Cleland WW. Dithiothreitol, a new protective reagent for SH groups. Biochemistry. 1964; 3: 480-482.
4. Reep BR, Kaplan PH, Kaplan, W.The use of n-acetyl-l-cysteine and dithiothreitol to process sputa for mycological and fluorescent antibody examination. Health Lab Sci. 1972; 9: 118-124.

SPUTAFLUID REF 224001
SDS rev 3
Regulation (EU) 2020/878

Contains: Na_2HPO_4 / dithiothreitol

Classification

Skin irritation, category 2	H315	Causes skin irritation.
Eye irritation, category 2	H319	Causes serious eye irritation.

Labelling
Pictogram

Signal word	Warning
Hazard statements:	
H319	Causes serious eye irritation.
H315	Causes skin irritation.

Precautionary statements:

P264	Wash hands thoroughly after handling
P280	Wear protective gloves/protective clothing/eye protection/face protection
P337+313	If eye irritation persists, consult a doctor.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. continue rinsing.

Contains: Na_2HPO_4 / Dithiothreitol

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	This side up	
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Revision 0	First edition	2022/05
Revision 1	Update of the Safety Data Sheet	2023/10

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.