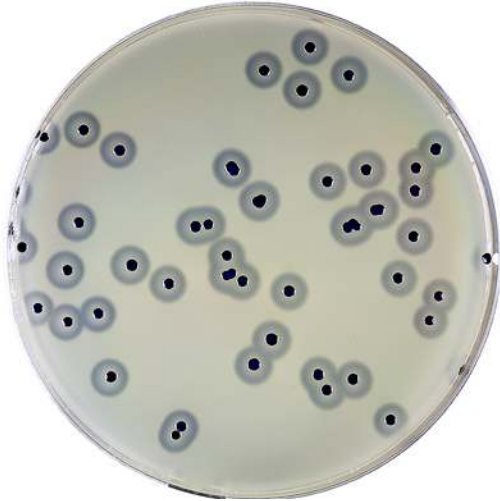




## BAIRD PARKER AGAR BASE BAIRD PARKER EGG YOLK TELLURITE AGAR BAIRD PARKER RPF AGAR

Dehydrated and ready-to-use culture media

Baird Parker Egg Yolk Tellurite Agar: colonies of *S. aureus*Baird Parker RPF Agar: colonies of *S. aureus* and *S. epidermidis*

### 1 - INTENDED USE

Baird Parker Agar Base, supplemented with Egg Yolk Tellurite Emulsion or RPF Supplement, is used for the enumeration of coagulase-positive staphylococci in foods and in other samples.

### 2 – COMPOSITION\*

#### BAIRD PARKER AGAR BASE – DEHYDRATED MEDIUM

##### TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Enzymatic digest of casein	10.00 g
Meat extract	5.00 g
Yeast extract	1.00 g
Sodium pyruvate	10.00 g
Glycine	12.00 g
Lithium chloride	5.00 g
Agar	15.00 g

#### BAIRD PARKER EGG YOLK TELLURITE AGAR - READY TO USE PLATES

Enzymatic digest of casein	10.00 g
Meat extract	5.00 g
Yeast extract	1.00 g
Sodium pyruvate	10.00 g
Glycine	12.00 g
Lithium chloride	5.00 g
Agar	15.00 g
Egg yolk emulsion	50 mL
Potassium tellurite 1%	10 mL
Purified water	1000 mL

#### BAIRD PARKER RPF AGAR - READY TO USE PLATES

Enzymatic digest of casein	10.00 g
Meat extract	5.00 g
Yeast extract	1.00 g
Sodium pyruvate	10.00 g
Glycine	12.00 g
Lithium chloride	5.00 g
Agar	15.00 g
Fibrinogen	3.8 g
Trypsin inhibitor	25.0 mg
Rabbit plasma (EDTA)	25.0 mL
Potassium tellurite	25.0 mg
Purified water	975 mL

\*The formula may be adjusted and/or supplemented to meet the required performances criteria.

### 3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Staphylococci are Gram-positive, non-motile, non-sporeforming, facultative anaerobic, catalase-positive cocci, with spherical cells 0.5-1 µm in diameter, occurring as single cocci, in pairs, or tetrads, or as short chains.<sup>1</sup> Staphylococci are part of the resident microbiota of mammals and birds, and their major habitats are the skin and mucous membranes.<sup>1</sup> Some species of staphylococci can cause a wide variety of infectious, usually pyogenic, processes in various parts of the body in animals and humans. Coagulase-positive staphylococci, especially *S. aureus*, through the production of enterotoxins, induce gastroenteritis following the consumption of contaminated food.

A selective and diagnostic medium for the enumeration of *S. aureus* in foods was first reported by Baird-Parker in 1962.<sup>2</sup> This medium is now widely recommended by international authorities for enumeration of coagulase positive staphylococci in foods and other materials of sanitary importance.<sup>3-9</sup>

In Baird Parker Agar, the enzymatic digest of casein, meat extract and yeast extract provide nitrogen, carbon, group B vitamins and minerals for microbial growth; sodium pyruvate is a critical component essential to both recovery of damaged *S. aureus* and their subsequent growth.<sup>2,3</sup> Selectivity is attained with lithium chloride, glycine and potassium tellurite which inhibit most bacteria present in the samples except coagulase-positive staphylococci. Selectivity can be improved by the addition of sulphamethazine for suppressing growth and swarming of *Proteus*.<sup>6,10,11</sup>

Egg yolk is the substrate to detect lecithinase and lipase activities: the egg yolk clearing reaction due to lecithinase is the diagnostic feature of characteristic colonies of *S. aureus*; an opaque zone of precipitation may form within the clear halo due to lipase activity. Rabbit plasma, fibrinogen, trypsin inhibitor are the substrates for detection of coagulase enzyme directly on the Baird Parker RPF plates.

Baird Parker Agar Base with Egg Yolk Tellurite Emulsion conforms to the formulation indicated by ISO 6888-1<sup>6</sup> and by FDA BAM<sup>5</sup>, while with RPF Supplement corresponds to the medium recommended by ISO 6888-2<sup>7</sup> and by ISO 6888-1 too only as an alternative to the coagulase test for confirmation. Both media are recommended by ISO 6888-3<sup>8</sup> for the MPN-method determination of low number of coagulase-positive staphylococci after enrichment in Giolitti and Cantoni Broth.



**4A - DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)****Egg Yolk Tellurite Medium**

Suspend 58 g in 1000 mL of cold purified water; heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and, using aseptic conditions, add 50 mL of Egg Yolk Tellurite Emulsion 20% (REF 423700); mix well and pour into sterile Petri dishes.

**Rabbit Plasma Fibrinogen Medium**

Suspend 5.8 g in 90 mL of cold purified water; heat to boiling with frequent agitation, sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and, using aseptic conditions, add the contents of one vial of RPF Supplement II (REF 423102) reconstituted with 10 mL of sterile purified water; mix well and pour into sterile Petri dishes.

**4B - DIRECTIONS FOR MEDIUM PREPARATION (READY TO USE FLASKS REF 5111162)**

Dissolve the contents (100 mL) of one flask of Baird Parker Agar Base in a water bath at 100°C. Cool to 47-50°C and, add 5 mL of Egg Yolk Tellurite Emulsion 20%, using aseptic conditions. Mix well and pour into sterile Petri dishes.

**4C - DIRECTIONS FOR MEDIUM PREPARATION (READY TO USE FLASKS REF 5131022)**

Dissolve the contents (90 mL) of one flask of Baird Parker Agar Base in a water bath at 100°C. Cool to 47-50°C and, add the contents of one vial of RPF Supplement II reconstituted with 10 mL of sterile purified water, using aseptic conditions. Mix well and pour into sterile Petri dishes.

**5 - PHYSICAL CHARACTERISTICS****Baird Parker Agar Base**

Dehydrated medium appearance straw coloured, fine, homogeneous, free-flowing powder

**Baird Parker Agar Base**

Prepared medium appearance yellow, limpid

**Baird Parker Egg Yolk Tellurite Agar**

Prepared plates appearance yellow, uniformly opaque

**Baird Parker RPF Agar**

Prepared plates appearance beige, slightly opalescent

Final pH at 20-25 °C

7.2 ± 0.2

**6 - MATERIALS PROVIDED**

Product	Type	REF	Pack
Baird Parker Agar Base	Dehydrated medium	4011162 4011164	500 g (8.6 L) 5 Kg (86 L)
Baird Parker Agar Base	Ready to use flasks	5111162	6 x 100 mL
Baird Parker Agar Base+ RPF Supplement II	Ready-to-use flasks and supplements	5131022	4 x 90mL Baird Parker flasks + 4 vials of RPF Supplement II (each for 90 mL of medium base)
Baird Parker Egg Yolk Tellurite Agar	Ready to use medium in plates	541116	2 x 10 plates ø 90 mm
Baird Parker Egg Yolk Tellurite Agar	Ready to use medium in plates	491116	3 x 10 plates ø 55 mm
Baird Parker RPF Agar	Ready to use medium in plates	543101	2 x 10 plates ø 90 mm
Baird Parker Egg Yolk Tellurite Agar	Ready to use medium in plates	501116P	5 plates ø 150 mm

The following products are available for the detection of coagulase-positive staphylococci, for which please refer to the specific instructions for use.

Egg Yolk Tellurite Emulsion 20%	Liquid supplement	423700 423701 423702	50 mL 100 mL 200 mL
RPF Supplement II	Freeze-dried supplement	423102 423102D	4 vials of 10 mL, each for 100 mL of medium 4 vials of 20 mL, each for 200 mL of medium
Coagulase Plasma EDTA CND: W0104080299, EDMA: 14.02.02.90, RDM: 1753966/R	Identification reagent	429936	4 vials with 5 mL of rabbit plasma → (4 x 15 mL: 120 tests)
Coagulase Plasma EDTA CND: W0104080299, EDMA: 14.02.02.90, RDM: 1753942/R	Identification reagent	429937	4 vials with 2.5 mL of rabbit plasma → (4 x 7,5 mL: 60 tests)
Coagulase Plasma EDTA CND: W0104080299, EDMA: 14.02.02.90, RDM: 1753964/R	Identification reagent	429938	10 vials with 1 mL of rabbit plasma → (10 x 3 mL: 60 tests)

**7 - MATERIALS REQUIRED BUT NOT PROVIDED**

Autoclave, water-bath, sterile loops, swabs and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, ancillary culture media and reagents.

**8 – SPECIMENS**

Materials of sanitary importance such as products intended for human consumption and the feeding of animals, environmental samples in the area of food production and food handling, cosmetics, water. Consult the appropriate references for sample collection, storage and preparation.<sup>4-9</sup>

**9 - TEST PROCEDURE**

**ISO 6888-1** recommends the procedure summarised below with Baird Parker Egg Yolk Tellurite Agar medium.

- Transfer by means of a sterile pipette 0.1 mL of the test sample if liquid or 0.1 of the initial suspension in the case of other products, to each of two agar plates. Repeat the procedure for further decimal dilutions if necessary.
- Carefully spread the inoculum as quickly as possible over the surface of the agar plate. Allow the plates to dry.
- Invert the plates and incubate them for 24 ± 2 hours at 34-38°C and re-incubate for a further 24 ± 2 hours.





4. After incubation for 24 hours mark on the bottom of the plates the positions of any typical colonies. Re-incubate, then mark any new typical colonies. Also mark any atypical colonies present.
  5. For enumeration, only retain plates containing a maximum of 300 colonies in total (typical, atypical, background flora), and including a maximum of either 150 typical or atypical colonies, or both, at two successive dilutions.
  6. Select for confirmation five characteristic colonies if there are only characteristic colonies, or five non-characteristic colonies if there are only non-characteristic colonies, or five characteristic and five non-characteristic colonies if both types are present, from each plate.
- ISO 6888-2** recommends the procedure summarised below with Baird Parker RPF Agar medium:
1. Transfer, by means of a sterile pipette, 1 mL of the test sample if liquid, or 1 mL of the initial suspension in the case of other products, to a Petri dish. Repeat the procedure for further decimal dilutions if necessary.
  2. Into each Petri dish, immediately pour 18 mL to 20 mL freshly prepared complete Baird Parker RPF Agar to obtain a depth of at least 3 mm.
  3. Carefully mix the inoculum with the culture medium and leave to solidify by placing the Petri dishes on a horizontal surface.
  4. After complete solidification, invert the dishes and incubate at 34 °C to 38 °C.
  5. After incubation for 24 h ± 2 h, mark on the bottom of the plates the positions of any typical colonies present. If no colonies or no typical colonies are obtained at 24 h ± 2 h, re-incubate all plates at 34°C to 38 °C for a further 24 h ± 2 h (to a total of 48 h ± 4 h), and mark any typical colonies.
  6. At the end of the incubation period, count the typical colonies in each dish. For enumeration, only retain plates containing a maximum of 300 colonies, with 100 typical colonies. One of the plates shall contain at least 10 colonies.
- Following the procedure for enumeration and detection by MPN given by **ISO 6888-3**, inoculate Baird Parker Egg Yolk Tellurite Agar or Baird Parker RPF Agar plates by subculturing the selective enrichment in Giolitti & Cantoni Broth (REF 401516).  
For spreading, preparation of the inoculated plates and incubation follow the instructions as given above.

## 10 - READING AND INTERPRETATION

### Baird Parker Egg Yolk Tellurite Agar

Typical colonies are black or grey, shining and convex (1 mm to 1.5 mm in diameter after incubation for 24 h ± 2 h, and 1.5 mm to 2.5 mm in diameter after incubation for 48 h ± 4 h) and are surrounded by a clear zone, which can be partially opaque. After incubation for at least 24 h, an opalescent ring immediately in contact with the colonies can appear in this clear zone.

Atypical colonies have the same size as typical colonies and can present one of the following morphologies:

- shining black colonies with or without a narrow white edge; the clear zone is absent or barely visible and the opalescent ring is absent or hardly visible;
- grey colonies free of clear zone.

The confirmation of coagulase positive staphylococci is undertaken by a coagulase tube test. Alternatively, it can be undertaken by a plate test using Baird Parker RPF Agar.

### Baird Parker RPF Agar

Typical colonies are black or grey or even white, small and are surrounded by an opacity halo of precipitation, indicating coagulase activity. Proteus colonies can appear to look like those of coagulase-positive staphylococci early on in the incubation. However, they can be distinguished from staphylococci after 24 h ± 2 h and 48 h ± 4 h of incubation, as their colonies become more or less brownish and start to spread. As the Baird Parker RPF Agar medium is based on a coagulase reaction, it is not necessary to confirm this activity.

## 11 - 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. Here below are listed some test strains useful for the quality control of Baird Parker Egg Yolk Tellurite Agar and Baird Parker Egg Yolk RPF Agar.<sup>6,7</sup>

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<b>Baird Parker Egg Yolk Tellurite Agar</b>		
<i>S. aureus</i> ATCC 6538	36 ± 2 °C / 24 ± 2 h / A	growth, black or grey colonies, surrounded by a zone of clearing
<i>S. saprophyticus</i> ATCC 15305	36 ± 2 °C / 48 ± 4 h / A	growth, black or grey colonies, without the clearing zone
<i>E. coli</i> ATCC 25922	36 ± 2 °C / 48 ± 4 h / A	inhibited
<b>Baird Parker RPF Agar</b>		
<i>S. aureus</i> ATCC 6538	36 ± 2 °C / 24 ± 2 h / A	growth, black or grey colonies, surrounded by an opaque zone
<i>S. saprophyticus</i> ATCC 15305	36 ± 2 °C / 48 ± 4 h / A	growth, black or grey colonies, without the opaque zone
<i>E. coli</i> ATCC 25922	36 ± 2 °C / 48 ± 4 h / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

## 12 – PERFORMANCE CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated Baird Parker Agar Base, supplements (Egg Yolk Tellurite Emulsion and Rabbit Plasma Fibrinogen Supplement) and ready to use plates and flasks are tested for productivity, specificity and selectivity by comparing the results with Tryptic Soy Agar.

The productivity is tested by a quantitative method with the target strains *S. aureus* ATCC 6538 and ATCC 25923: the plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 35-37°C for 18-24 hours. The colonies are enumerated on both media and the productivity ratio (Pr) is calculated. If Pr is ≥ 0.5 and if the colonies morphology and colour are typical (black or grey colonies, surrounded by a zone of clearing or black or grey colonies, surrounded by an opaque zone) the results are considered acceptable and conform to the specifications.

The specificity characteristics are tested by semi-quantitative ecometric technique with the following target strains: *S. saprophyticus* ATCC 15305 and *S. epidermidis* ATCC 12228. After incubation, the amount of growth and the colony characteristics are evaluated: both strains exhibit growth, with black or grey colonies without halos.

The selectivity is assessed with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of *E. coli* ATCC 8739 and *E. faecalis* ATCC 19433. The growth non-target strains is totally inhibited.

## 13-LIMITATIONS OF THE METHODS

- The methods described here are intended to be applicable to the detection and enumeration of coagulase-positive staphylococci among which enterotoxigenic strains are encountered. It is mainly concerned with *S. aureus*, but also with *S. intermedius* and certain strains of *S. hyicus*.<sup>6-8</sup>





- The confirmation of typical and atypical colonies is based on a positive coagulase reaction, but it is recognized that some strains of *Staphylococcus aureus* give weakly positive coagulase reactions. These latter strains can be confused with other bacteria but they can be distinguished by the use of additional tests.<sup>6-8</sup>
- Colonies with typical appearance after 24 h ± 2 h incubation on Baird Parker Egg Yolk Tellurite Agar can lose their typical appearance after 48 h ± 4 h incubation, due to overgrowth with enlargement of the clear zone during the second phase of incubation. Counting only at 48h ± 4h can lead to low counts or no counts.<sup>6,8</sup>
- Even if there is an inhibitor of trypsin in the Baird Parker RPF medium, colonies with typical appearance after 24 h ± 2 h incubation can lose typical appearance after 48 h ± 4 h incubation, due to enzymatic processes (trypsin) or due to overgrowth. Counting only at 48 h ± 4 h can lead to low counts or no counts.
- Bacteria belonging to genera other than staphylococci can give colonies with an appearance similar to staphylococci on Baird Parker Egg Yolk Tellurite Agar. Microscopic examination of Gram stain, before confirmation, will enable the distinction of other genera from staphylococci.<sup>6-8</sup>
- On Baird Parker RPF Agar, *Proteus* colonies can appear to look like those of coagulase-positive staphylococci early on in the incubation. However, they can be distinguished from staphylococci after 24 h ± 2 h and 48 h ± 4 h of incubation, as their colonies become more or less brownish and start to spread.<sup>7</sup>
- Occasionally other organisms exhibit growth without typical reactions on Baird-Parker Agar, e.g., some strains of streptococci, micrococci, corynebacteria and enterobacteria, some yeasts, fungi and bacilli that are easily distinguishable by the morphology and grey colour of the colonies.<sup>11</sup>

#### 14 - PRECAUTIONS AND WARNINGS

- The products here described are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplements shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media must be handled with suitable protection. Before use, consult the Material Safety Data Sheets.
- This culture medium contains raw materials of animal origin. The ante and post mortem controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website [www.biolifeitaliana.it](http://www.biolifeitaliana.it), describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Be careful when opening screw cap flasks to prevent injury due to breakage of glass. Be careful when opening the metal ring to avoid injury.
- When using a hot plate and/or a water bath, boil sufficiently long to dissolve the whole medium.
- Wear heat-protective gloves during medium liquefaction. Do not place the hot flasks into an ice bath or in cold water to accelerate cooling as this might cause cracks in the glass.
- The time required for complete liquefaction of the medium may vary considerably and depends on the actual temperature of the heating device, its wattage, the size and volume of the bottle.
- Once the bottled medium is liquefied, it cannot be solidified and dissolved a second time.
- Ready-to-use flasks are subject to terminal sterilization by autoclaving.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet are available on the website [www.biolifeitaliana.it](http://www.biolifeitaliana.it).
- 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.

#### 15 - STORAGE CONDITIONS AND SHELF LIFE

##### Ready to use plates

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

##### Ready-to-use medium in flasks

Upon receipt, store flasks in their original pack at 2-8°C away from direct light. If properly stored, the flasks may be used up to the expiration date. Do not use the flasks beyond this date. Flasks from opened secondary packages can be used up to the expiration date. Opened flasks must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use flasks with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

##### Dehydrated medium

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/bottles) and the applied storage conditions (temperature and packaging). According to ISO 6888-1, Baird





Parker Agar Base may be store in flask for up to 6 months at 2-8°C, while the prepared Baird Parker Egg Yolk Agar and Baird Parker RPF Agar plates may be stored, prior to drying, at 2-8°C for up to 14 days.<sup>6</sup>

### 16 - REFERENCES

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6. ISO 6888-1:2021. Microbiology of the food chain - Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species)-Part 1: Method using Baird-Parker agar medium.
7. ISO 6888-2:2021. Microbiology of the food chain - Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) -Part 2: Method using rabbit plasma fibrinogen agar medium.
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9. ISO 22718 Second edition 2015-12-01 Cosmetics — Microbiology — Detection of Staphylococcus aureus
10. Smith BA, Baird-Parker AC. The use of sulphamezathine for inhibiting Proteus spp. on Baird-Parker's isolation medium for Staphylococcus aureus. J Appl Bact 1964; 27: 78-82.
11. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
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### TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature imitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

### REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2022/07

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

