BIOBALL® LUMINATE 2.0

Frequently Asked Questions

What is BIOBALL® LUMINATE 2.0?

BIOBALL® LUMINATE 2.0 are small freeze-dried water-soluble balls containing a precise number of viable microorganisms. Each BIOBALL® LUMINATE 2.0 is tagged with a Green Fluorescent Protein (GFP) marker integrated into the chromosome. They are presented in small glass tubes with a secure cap, in a single format of 10 vials, each containing 1 ball at a precise concentration of 100 cfu (with a SD of 15%).

For the complete description, please refer to the webpage of BIOBALL® LUMINATE 2.0:
BIOBALL® Standardized Strains for Food Applications | Pioneering Diagnostics (biomerieux.com)

Why 100 cfu?

This precise concentration is particularly suitable for the main applications in food labs for quality control, by only making dilutions:

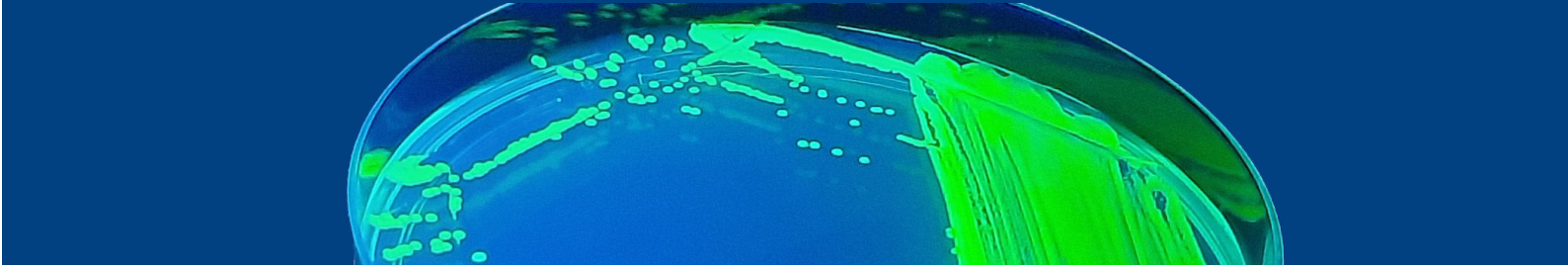
- Positive quality controls (usually 5 tests/BIOBALL®)
- Verification of methods (1 verification/BIOBALL®)

This precise count limits the handling of strains, avoids culture and optimizes the cost per test.

What are the available BIOBALL® LUMINATE 2.0 strains?

The BIOBALL® LUMINATE 2.0 range consists of five strains, all ISO 11133 compliant and derived from NCTC culture collections:

- 423938 *Salmonella* Typhimurium (from NCTC 12023 - WDCM 00031)
- 423939 *Escherichia coli* O157:H7 (from NCTC 12900 - WDC M00014)
- 423940 *Cronobacter sakazakii* (from NCTC 11467 - WDCM 00214)
- 423941 *Listeria monocytogenes* 4b (from NCTC 10527 - WDCM 00021)
- 423942 *Listeria innocua* (from NCTC 11288 - WDCM 00017)



Why using BIOBALL® LUMINATE 2.0?

The use of collection strains for quality control increases the risk of cross-contamination, leading to false positive results on tested samples. To better manage this risk, food labs can use standardized and ready-to-use collection strains, just like BIOBALL® LUMINATE 2.0. Because they are genetically modified microorganisms (GMM), they are moreover easily distinguishable from natural contaminants which facilitates the confirmation of positive results of food samples.

To learn more about cross-contamination in food laboratories, please refer to our dedicated scientific article: Control the Risk of Cross-contamination in Food Microbiology Laboratories | Pioneering Diagnostics (biomerieux.com)

What is the protocol for quality control of methods?

After a simple re-hydration (with 1,1 mL of 14-day re-hydration fluid), aliquots can be prepared at the desired concentration and be frozen. The sample used to control the method is then diluted into culture media and seeded with a precise count of fluorescent bacteria. After incubation, the method is performed according to the package inserts and positive results are expected. This protocol has been successfully evaluated on the most sensitive bioMérieux detection methods.

To learn more about our evaluations, please refer to our poster P2-33 (IAFP 2023) :
How to implement the use of genetically modified microorganisms for routine quality control of methods in labs?

To watch the video of the protocol: https://youtu.be/L9e9boJx_ys

What is the protocol for verification of methods?

A protocol for verification of methods (e.g., according to ISO 16140-3 or 16140-4) requiring a precise artificial contamination can be implemented by using BIOBALL® LUMINATE 2.0. The protocol 3 from ISO 16140-3 has been successfully evaluated on the most sensitive bioMérieux detection methods, as presented in the poster P2-33 (IAFP 2023) linked above.
If support is needed, please contact your bioMérieux representative who will provide you with a tailored service offer.

To watch the video of the protocol: https://youtu.be/O9zMQYRR-bY

Is it possible to use BIOBALL® LUMINATE 2.0 straight from the freezer?

Once re-hydrated, BIOBALL® LUMINATE 2.0 can be used right away, or stored in the freezer. In that case, they can be used later, straight from the freezer, with no additional preparation required.

What are the shelf life and temperature of storage of BIOBALL® LUMINATE 2.0?

BIOBALL® LUMINATE 2.0 strains have a shelf life of 1 year* (minimum 4.5 months at shipping) or 2 years (minimum 6 months at shipping) after the date of manufacture, depending on the strain. Because of their specific production process to have a highly precise count, they must be stored between -18°C and -33°C.

Is it possible to cultivate BIOBALL® LUMINATE 2.0?

As any strains, BIOBALL® LUMINATE 2.0 can be cultivated by following standard culture protocols. However, each ready-to-use ball contains a precise count of microorganisms, thus avoiding the cultivation step and its inherent cross-contamination risks.

Are the BIOBALL® LUMINATE 2.0 strains stable after several passages?

Due to the chromosomic insertion of new generation, these strains are highly stable and fluorescent, even after multiple sub-cultures (passages). The stability of the fluorescence has been validated up to 4 passages (in addition to those initial) which is enough for detection methods. It is not recommended to cultivate BIOBALL® LUMINATE 2.0 for quality control to limit the risk of cross-contamination and keep a precise count of microorganisms.

To learn more about our evaluations, please refer to our poster P2-33 (IAFP 2023) :
How to implement the use of genetically modified microorganisms for routine quality control of methods in labs?

Are they usable according to ISO 11133?

BIOBALL® LUMINATE 2.0 are compliant with strains listed in ISO 11133 standard for performance testing of culture media and reagents. BIOBALL® LUMINATE 2.0 *Listeria monocytogenes* and *Listeria innocua* at 100 cfu are particularly adapted to test the performance of culture media for enumeration. They have been successfully evaluated (with one BIOBALL® + 100 µL of saline solution) on TSA, ALOA® and PALCAM agars.

How to be sure that a positive result on a tested sample is not due to cross-contamination?

When a positive result is obtained in a food lab, it can be due to cross-contamination with quality control strains. By using fluorescent strains for these quality controls, it is easy to confirm that a positive result is not due to control strains. A simple reading of the confirmation plate under UV light can confirm the absence of fluorescent QC strains. A more sophisticated technique using PCR, as the VERIFLOW® Green Fluorescent Protein, can also be used to confirm the absence of cross-contamination.

To watch the videos presenting these confirmation techniques:
- UV lamp confirmation: https://youtu.be/FNvs7s-KO2A
- VERIFLOW® GFP confirmation: https://youtu.be/BQXoPIE3yV0

Evaluations of confirmation techniques are also presented in the poster P2-33 (IAFP 2023) above.

Is there a need to apply for an official agreement before ordering and/or using BIOBALL® LUMINATE 2.0?

BIOBALL® LUMINATE 2.0 is a range of genetically modified microorganisms (GMM) and may need to comply with special regulatory requirements for laboratory use (e.g., European directive 2009/41/EC completed by national regulation). To declare or obtain an agreement before use, please refer to your local competent authority. It is the responsibility of the lab to apply for an agreement, if requested in their country. bioMérieux does not require proof of agreement to fulfill orders.

How to know if my country requires approval/agreement before using GMM?

bioMérieux can support you by providing information about the need and process for agreement for many countries. For any questions, please contact your local bioMérieux representative.

Does genetic modification change the biosafety level (class) of the host strains?

All the host strains are BSL-2 and the genetic modification does not change the biosafety level.
The question is often asked about Escherichia coli O157:H7. BIOBALL® LUMINATE 2.0 *Escherichia coli* O157:H7 (ref 423939) is derived from NCTC 12900 which is a non-toxigenic strain:

- Hazard Group (ACDP): 2
- Serotype O157:H7, verocytotoxin (shigatoxin (stx)) negative

Source: Bacteria Collection: NCTC 12900 *Escherichia coli* (culturecollections.org.uk)

Is the use of GMM accepted by official standards?

The use of genetically altered control strains for microbiological quality controls is now recommended at ISO/TC34/SC9 and CEN/TC463. As they are easily distinguishable from natural contaminants, their use enables labs to quickly and easily confirm real positive results, thus limiting the impact of cross-contaminations and false positive result release. This recommendation will be incorporated into the coming revision of ISO 7218 and potentially into the future revision of ISO 11133.

To learn more about this revision, please refer to our 2nd poster P2-31 (IAFP 2023):
Which QC strains to use for minimizing risk of false positives due to cross-contamination in a lab?

Are there other BIOBALL® LUMINATE 2.0 strains under development?

At bioMérieux, we are constantly looking for innovative solutions specifically designed for food industrials. Therefore, we first developed the five most impactful pathogenic strains used in food microbiology labs. Others may be developed if food industrials need them.

Are classical BIOBALL® available for food applications?

The classical non-fluorescent BIOBALL® can also be used in food labs for quality controls. All the available strains with their reference are listed on the webpage of BIOBALL® LUMINATE 2.0. Of course, their use must be evaluated before routine application.

Is it possible to order custom-made BIOBALL®?

bioMérieux offers a dedicated custom service to develop BIOBALL® strains in addition to those commercialized. These customized strains can be developed from new strains, strains provided by the requesting lab or existing BIOBALL® (classic or fluorescent, at another concentration). 12 weeks are necessary for the development of the custom-made strains, with a minimum order of 7,500€ or 100 vials (either 10 kits of 10 packs or 5 kits of 20 packs).

Are the BIOBALL® LUMINATE 2.0 strains CRM certified?

All BIOBALL®, including the Luminate 2.0, are Certified Reference Materials (ISO 17034). They come with a Certificate of Analysis detailing the strains identity, characteristics and their precise count with standard deviation.

*The 1-year shelf life for *Listeria*, *Cronobacter* and *E. coli* will be extended to 2 years

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