Condagene®

Condagene® ViableCell Reagent (CAT. 6502)

Purpose, features and context

Purpose

This reagent is intended to neutralize DNA from dead cells and/or free DNA that could lead to false positives in PCR-based detection techniques. This reagent cannot cross entire cell membranes associated with cell viability.

The results obtained from PCR in samples treated with Condagene® ViableCell are indicators of the presence of viable cells.

Features and Context

Condagene® ViableCell is added to a suspension of cells in PBS or Ringer's 1/40 at a final concentration of 25-100 µM. It is left in contact with the reagent for 15-30 min in the dark, after which time the sample is photoactivated with blue light (e.g., miniblue) for 15 minutes. The DNA that has come into contact with the reagent is covalently bound to it, ceasing to be a suitable template for PCR, so only the DNA of viable cells will be detected.

Both the treatment protocol and the PCR kit used significantly impact the method's performance.

Instructions for use

Content

This product contains 2 vials with 0.5 mg of Condagene® ViableCell.

Additional equipment required

- Vortex, or tube mixers
- Variable volume micropipette
- Filter pipette tips
- Thermal block with agitation, optional
- High transparency, low affinity DNA binding microcentrifuge tubes
- Tube Rack
- Photoactivation device

Storage and preservation

This product is shipped at room temperature. In the fridge (3-5°C), it will remain stable for at least one year. It can be tough for at least 6 months in an aqueous solution and under refrigeration.

This reagent is photosensitive. Avoid contact with light.

Microbiological state

Sterile products.

Preparation of reagents

Add 0.490 mL of sterile distilled water to a Condagene® ViableCell tube to achieve a final concentration of 2000 µM.

General instructions

Carefully read the safety data sheets for this and all the products that you will use throughout the workflow.

This product is manufactured and sold for quality control in food / environmental samples, it has not been designed for any other use, especially for the diagnosis of infectious diseases. Its use must be carried out by experienced and qualified personnel in the handling of potentially dangerous chemical products.

Users must make independent decisions about the integrity of the information based on all available sources. Manufacturer is not responsible for any damage caused by handling or contact with the product.

If the Condagene® ViableCell concentration range will be <50 µM or you will work with sample volumes less than 1 mL, it may be convenient to prepare working solutions with a dilution factor of 1/10.

Operation procedure

Actions to be performed before starting

All PCR workflows must be carried out under conditions that prevent environmental contamination. It is recommended that in each series you include at least one blank to keep contamination risks under control.

- Remove the reagent from the refrigerator
- Start the thermal block, and set the temperature to 30-37°C

The protocol begins on a pellet of the sample, obtained by centrifuging for at least 10-13,000 g for 5-10 minutes, which can have a diverse nature (see table 1).

Resuspend this pellet in 0.5 mL of PBS or Ringer's 1/40.

Table 1. Sample examples to process

TYPE	AMOUNT
Wastewater	Concentrate from 100-50 mL
Drinking Water	Concentrate from 1 L
Continental Water	Concentrate from de 0,5-1 L
Enrichment Broth	≤1 mL

Protocol

1. Add the reagent (Condagene® ViableCell 2000 µM) for example according to the following table:

SAMPLE	µL REAGENT	µM reagent
0,5 mL	6,25	25
0,5 mL	12,5	50
0,5 mL	25	100

Note: The performance of the Condagene® ViableCell function can be improved with the additional dosage of compounds such as SDS, Deoxycholate or in some cases with PEG. The use of these compounds is not universal, it must be done minimizing the impact it may have on viable cells.

2. Vortex shake for 30 seconds.

3. Incubate at room temperature or 37°C for 30 min in the dark.

Note: The free DNA present in the sample may adhere to the walls of the microtubes, making it difficult for the reagent to access it due to electrostatic repulsion. Some authors recommend using low-bind DNA tubes and even transferring the content to a new tube before the photoactivation step.[1]

4. Photoactivate the tubes, e.g., 15 min on blue led devices.

5. Centrifuge the sample 10,000 g for 10 minutes and remove 450 µL of supernatant.

The sample is ready to proceed with DNA extraction.

Trademarks and licenses

The use of this product may be covered by Licenses, patents or pending patent applications belonging to manufacturer. Customers who received this product may use it for research and quality assessment purposes in food/environmental samples without infringing intellectual property rights.

In some cases, such as the combination of Condagene® ViableCell with PEG and the like, in equimolar concentrations, it may be subject to industrial property rights belonging to third parties. The purchase of this reagent does not include any transfer of rights of use from third parties.

The use of this product is not intended for therapeutic purposes, domestic, agricultural, or cosmetic use. Its use must be supervised by a technically qualified person with experience in handling potentially dangerous chemical products. Users must make independent decisions about the integrity of the information based on all available sources.

Manufacturer is not responsible for any damage resulting from handling or contact with this product.

[1] Agusti, Gemma & Fittipaldi, Mariana & Codony, Francesc. (2017). False-Positive Viability PCR Results: An Association with Microtubes. Current Microbiology. 74. 10.1007/s00284-016-1189-3.

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If you need more information about qPCR products and techniques for pathogen detection, do not hesitate to contact us.