



# FISH-kit™

Reliability

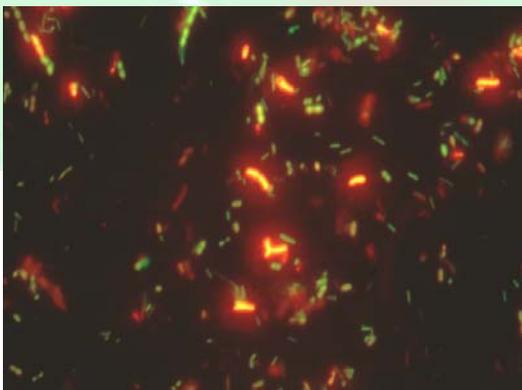
Speed

Easy

## Fluorescence *in situ* Hybridization (FISH)

The detection of whole-bacterial cells via the labelling of specific nucleic acids with fluorescently labelled oligonucleotide probes is called fluorescence *in situ* hybridization (FISH). FISH requires no cultivation and cells can be fixed before analysis. Compared to conventional cultivation techniques this method offers five distinct advantages:

- 1) FISH allows the detection of one to three orders of magnitude more bacterial cells in samples. Even when using optimal media and growth conditions, generally less than 1-10% of the bacteria present in life samples will develop into detectable colonies.
- 2) FISH allows the study of the actual composition of the microbial community. When using cultural methods, over-representation as well as under-representation of populations can occur due to selectivity of the applied cultivation conditions.
- 3) FISH allows the *in situ* localisation and the study of spatial organisation of bacterial cells as they occur in their natural habitat.
- 4) Unlike immunological methods for detection, FISH is not dependent on extracellular macromolecules that may only be expressed under certain cultivation conditions, but that are absent in other situations. FISH results are always definitive.
- 5) For FISH, cells need not be alive. Samples can easily be fixed and collected for later analysis. The intensity of the fluorescence is a direct measure for the activity of the cells. Inactive cells can be recognised by their low intensity fluorescence.



## Ribo Technologies FISH Kit for detection of bacteria.

The FISH assay is a culture-independent technique and has been proven to be a reliable and validated method for the enumeration of whole bacterial cells in mixed populations<sup>7</sup>. At Ribo Technologies, we have dedicated ourselves to the validation and optimisation of this methodology. Much of our R&D work is assembled in the FISH Kit. The FISH Kit combines the advantages of FISH technology with the ease and reliability of standardised assays.

## The many features of the Ribo Technologies FISH Kit

### **Very rapid results**

The Ribo Technologies FISH Kit is very rapid. Results can be read in under 24 hours after sample collection!

### **Strong signals**

In order to achieve outstanding fluorescence signals, the Ribo Technologies FISH Kit combines optimal target selection with state-of-the-art fluorescent labelling techniques.

### **Validated Controls**

At Ribo Technologies we excel in the art of validation. Empirical testing of a large number (>35) of reference cultures, and an elaborate testing program have successfully been passed before we were satisfied with this system.

### **Easy protocols**

Since your ease is our effort, we have attempted to produce a system that is extremely robust and straightforward in use and at the same time easy to interpret.

### **Convenient storage**

The oligonucleotides in the hybridization mixture are stable for up to 6 months when stored in the dark at room temperature. For some bacteria a permeabilization formula is necessary. The permeabilization formula, permeabilization buffer and the mounting fluid are stable when stored at 4 °C. Immersion oil should be stored in the dark at room temperature.

**Fluorescence *in-situ* Hybridization using the EASYprotocol™**

The enclosed **EASYprotocol™** was developed for the identification of bacteria by fluorescence in situ hybridization (FISH) using the Ribo Technologies FISH Kit. This protocol should be performed by suitably skilled laboratory personal. Before starting, read the entire protocol carefully, and take appropriate precautions when using hazardous substances. The results can be evaluated within 24 hours. Note that the morphology of the cells may alter slightly due to the various treatments.

**Comments and Suggestions**

We invite you to inform us about any comments or suggestions regarding the use and performance of this test kit. In case the product did not reach you in proper condition or if you are not satisfied with the product for any other reason, notify us immediately.

Below a short selection of our FISH testkits is given. FISH test can also be tailor-made with a development time of 1-2 months.

**FISHkit™**

A range of kits for the research Market for Functional Food/Feed/Pharma Gutflora Analysis (FISH on Fluorescence Microscope).

The test kits are available in different formats;

ME, Microscopic enumeration  
MC, Microscopic confirmation  
FE, Flowcytometric enumeration

**Other equipment required**

In order to work with the Ribo Technologies FISH Kit you need several items yourself. Most of these items belong to the standard inventory of modern microbiological laboratories. The following is needed to complete the protocol and visualize the cells microscopically.

- An epifluorescence microscope fitted with a 100 W Mercury or Xenon arc lamp and filter sets for FITC (excitation 495 nm; emission 520 nm) and/or Cy3 (excitation 550 nm; emission 570 nm)
- A microcentrifuge ( 15,800 G)
- Adjustable pipettes for 10, 100 and 1,000 µl
- Pipette for 5 ml
- Source of double distilled water, Milli-Q or equivalent
- A stationary incubator at 40 - 60°C
- Filtration device (included: Spring clamp, glass funnel and afritted glas base)
- Filtering flask
- Vacuum/pressure pump
- Sterile glass-beads (diameter 3 mm)
- Supply of sterile microcentrifuge tubes (1.5 ml)
- A pair of tweezers

**A short selection of our FISH testkits**

Bifidobacterium	Genus
Bacteroides fragilis	Cluster
Bacteroides distasonis	Species
Escherichia coli	Species
Lactobacillus	Genus
Streptococcus	Genus
Bacteroides/Prevotella	Group
Clostridium butyricum	Group
Clostridium lituseburense	Group
Clostridium coccoides	Group
Clostridium difficile	Species
Enterococcus faecium	Species
Enetrococcus faecalis	Species
Leuconostoc mesenteroides	Species
Lactococcus lactis spp. lactis	Subspecies
Lactococcus lactis spp. cremoris	Subspecies
Streptococcus thermophilus	Species
Lactococcus lactis	Species
Staphylococcus spp./ CNS	Group

Manufactured by

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