FREQUENTLY ASKED QUESTIONS

FastOrange

What is the difference between FastOrange® B tubes and FastOrange ® B Ready-to-Use tubes?

Although the liquid in the tubes may look equal, there is a different broth in these two products.

FastOrange® B Tubes contain same broth as all other B products (bottles, enrichment tubes, and tubes) – therefore same range of microorganisms will grow.

Ready to Use Tubes – called Hygiene Swabs for the other product lines – are a bit different in ingredients and intended to monitor the overall hygiene status. Therefore, there is a wider range of microorganisms detected, for FastOrange® B Ready to Use tubes the hygiene indicator bacteria as *E. coli* – which won't grow in FastOrange® B broth.

Can I use FastOrange® for enrichment prior to PCR?

Sure you can, the whole FastOrange® product family was developed as pre-enrichment media for PCR users, but can also be used as stand-alone media for visual read-out of positives. We test each lot of FastOrange® by real time PCR which means there won't be inhibition or false positive results from FastOrange® enriched samples in your PCR test.

What kind of container size from your product range should I chose?

Small production places with no micro lab use the Tube size packages or the Enrichment Bottles, where they get sterile flasks pre-filled with the medium. They just add sample and incubate at ambient temperature. With these products, there is no need for sterilization, not even if you have your own lab.

Larger breweries with the capabilities to work in sterile conditions and sterilize their own flasks can use the bottles which delivers the broth in bulk glass bottles (240mL).

Is it possible to get growth in PIKA FastOrange Wild Yeast / BRETT, but negative results when following with PCR?

On Wild Yeast broth and agar not only Brettanomyces and S. diastaticus might grow, but also other wild yeast species. In our evaluations, we found positive growth for non-Saccharomyces yeasts *Pichia, Candida, Debaryomyces, Hanseniaspora, Torulaspora,* and *Zygosaccharomyces.* Besides, some of the British Ale yeasts may show visible growth, too. Therefore, it is generally recommended to further identify positive growth by PCR and / or test your brewery yeast strain for growing capabilities.

So far, only the *Brettanomyces* and *diastaticus* yeasts are described as typical beer spoilers, but from a more general perspective, the other yeasts are indicators for a bad hygiene status.

PIKA WEIHENSTEPHAN GmbH

P: +49 (0) 8441 879 48 30
E: order@pika-weihenstephan.de
W: www.pika-weihenstephan.de

Raiffeisenstrasse 31A,
85276 Pfaffenhofen,
GERMANY

Ref 2309E
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I have just started using your FastOrange broth for beer analysis. I have noticed that whenever I add the beer to the FastOrange the sample immediately turns significantly yellow/orange. Is this a positive test result?

The color change in PIKA FastOrange is depending on the pH value - if you analyze low pH products as sour beers, you might see an immediate color change when adding the sample. This is not an indicator for presence of spoilers.

Firstly, you could incubate such samples for a couple of days longer and watch for sediment and/or haze formation. Secondly, you could carry out a serial enrichment, meaning remove a part of the first enrichment after 2-3 days and enrich it again in fresh medium. This should solve the immediate color change problem and also will improve the detection limits for beer spoilers enormously.

If you are unsure after the incubation in liquid broth, you might be either do PCR analysis with our 4everyone Detection Kits to confirm the result, or you can do plating of the liquid enrichment after 5 days onto PIKA FastOrange® Agar - then you will clearly see colonies building up.

For ease of sample preparation and accuracy/consistency of results it would be ideal to be able to detect multiple organisms in tandem with each other. Is this possible?

Our DNA extraction protocol is equal for yeast and bacteria detection, so you can do one extraction per sample and then use the isolated DNA for different tests, both bacteria and yeast. Also, you can run all of our PCR tests side by side together in the thermocycler, as all our kits are optimized and follow the same PCR protocol.

I ran out of positive DNA before testing all 48 reactions. What am I doing wrong?

The positive control comes in the kits as a tube with 50 µl DNA, you need 5 µl per PCR run which means it lasts for 10 runs.

The thing is that in every PCR run you always use one extra tube as positive control. If you run two samples at a time, then you'll need 5 µl of control DNA per 3 PCR tubes – this means you'll need up to 80 µl control DNA for using one kit if analyzing always two samples at a time. In that case, the positive control DNA from one kit won't be sufficient. However, you can easily get an extra tube of control DNA as a spare part which you can order together with kits.

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