**WILD YEAST COPPER ADDITIVE <5%**

for Wild Yeast Detection with FastOrange® Yeast Bouillon glass bottles 240 ml

SKU #2050-2038-1

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.99% Copper Sulphate Stock Solution, sterile</td>
<td>12 x 4.8 mL</td>
<td>Store at ambient temperature</td>
</tr>
</tbody>
</table>

⚠️ **Warning!** Read the manual and the Safety Data Sheets before starting the analysis. Safety Data Sheets are online available at [www.pika-weihenstephan.com](http://www.pika-weihenstephan.com). All handling steps should be performed under sterile conditions. Wear appropriate protective clothing.

For *in vitro* use only.

**Product description**

Wild Yeast Copper Additive <5% is a chemical supplement which allows the detection of *Saccharomyces cerevisiae* var. diastaticus and other Wild Yeasts. The principle of its selectivity is based on the EBC method 4.2.5.1 *Saccharomyces Wild Yeasts, Cu-differentiation* which describes the use of nutrient media supplemented with copper sulphate to inhibit the growth of brewer’s yeast.

PIKA FastOrange® Yeast Bouillon is a culture medium developed to detect contaminations by yeasts, ref. to SKU #2038-1.

The combination of PIKA FastOrange® Yeast Bouillon and Wild Yeast Copper Additive <5% is optimized to quickly grow Wild Yeasts, while suppressing growth of most brewing yeasts.

**Guidelines for use**

A. **Preparation of Bouillon glass bottle**

1. One vial of Wild Yeast Copper Additive <5% is needed per Yeast Bouillon glass bottle.
2. Add one whole vial of Wild Yeast Copper Additive <5% into a FastOrange® Yeast Bouillon glass bottle and mix well.

**Important!** For *Saccharomyces cerevisiae* var. *diastaticus* analysis, a serial enrichment by the following method is necessary. Prepare a sufficient volume of Yeast Bouillon with copper sulphate before you start your enrichments.

B. **Enrichment of Samples**

1. Add an equal volume of FastOrange® Yeast Bouillon prepared with Wild Yeast Copper Additive <5% (as per A.) to your sample and mix. The final concentration of the medium will be ≥ 50%. The medium does NOT need to be accurately measured, it is sufficient to pour it and visually judge the volume.
2. For example, mix approx. 50 mL of sample with approx. 50 mL of broth
3. First enrichment: Incubate at 25 ± 2 °C for 1 day
4. For serial enrichment, discard half of the enriched sample volume (from 2. above).
5. Add an equal amount of prepared FastOrange® Yeast Bouillon with Wild Yeast Copper Additive <5% (as per A.) to the vessel containing the first enrichment (from 3.).
6. Second enrichment: incubate bottle from 3. above at 25 ± 2 °C for 1 day

**Incubation conditions**

We recommend the use of an enrichment vessel in a format that maximizes liquid surface to increase oxygen supply. Do not fully tighten the lid to allow gas exchange.

**Detectable microorganisms**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Growth conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>aerobic or anaerobic</td>
</tr>
<tr>
<td>var. <em>diastaticus</em> and <em>Brettanomyces</em></td>
<td></td>
</tr>
<tr>
<td>Other Wild Yeasts</td>
<td>aerobic or anaerobic</td>
</tr>
</tbody>
</table>

Growth of typical *Saccharomyces cerevisiae* brewing yeasts and of bacteria* is suppressed.
We recommend

1. Verify the presence of Wild Yeasts using PCR analysis. Refer to the list at the end of the manual for available PCR Detection Kits for yeast detection and/or identification.

2. Verification of liquid enrichments can be achieved by further enrichment on pour plates or by streaking out an aliquot on agar plates. We recommend the use of FastOrange® Wild Yeast Agar.

3. For growing colonies on the Wild Yeast Agar plate we recommend a microscopic examination.

* Growth of rare chloramphenicol resistant bacteria may appear.

** In our study of more than 200 brewing yeast isolates we observed that in rare cases colonies may form, particularly with some English Ale yeasts.

General information

Store the product at room temperature (max. 25°C). Cold storage below 25°C is NOT necessary.

Best before date for unopened products are given on the outer label. After opening we cannot guarantee the shelf life. The product is not suitable for human or animal consumption. It must not be used for the direct propagation of yeasts which are later used for food production or might get into contact with food.

FastOrange® Wild Yeast Products

Wild Yeast Agar (12 x 170 mL) SKU 2039-2
Wild Yeast Copper Additive <1% (15x 4 mL) SKU2050-2038-11
Wild Yeast Copper Additive <5% (12x 4.8 mL) SKU2050-2038-1

FastOrange® Yeast Products for preparation

Yeast Enrichment Bottles (15 x 40 mL) SKU 2038-11
Yeast Bouillon (12 x 240 mL) SKU 2038-1

4e® For everyone Detection Kits

Superattenuator Yeasts Screening SKU 2402-58
S. c. var. diastaticus Screening SKU 2402-49
Dekkera (Brettanomyces) sp. Screening SKU 2402-20
Dekkera (Brettanomyces) bruxellensis SKU 2402-54
Dekkera (Brettanomyces) anomala SKU 2402-55
Dekkera (Brettanomyces) naardenensis SKU 2402-56

**Notes:** The relevant antibiotics/fungicides contained in the medium fall short of critical values that require monitoring or declaration according to regulation (EG) 1907/2006 (REACH). When properly applied, the medium may be disposed of through the normal sewage system. It is strongly recommended to inactivate the live microorganisms in any enriched sample by heating to 121°C/250°F for 20 min (autoclave) to exclude a release of live microorganisms. Although this information was collected thoroughly, we cannot guarantee that any of the content is incomplete or incorrect. We do not take over any warranty for consequences which are resulting from improper handling or incorrect use of this product. Additionally, always comply with the applicable laws, regulations and directives of your country.

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