Chai + PIKA
Brewing Quality Solutions

Running your PIKA 4e Kit

Welcome to Chai + PIKA Brewing Quality Solutions. This manual will walk through the protocol for using PIKA test kits for beer spoiler detection and is intended as a general kit guide.

Please consult the individual kit manuals and enrichment guides for complete details on sample enrichment and kit protocol.

Contents

I: Enrichment or Direct qPCR?

First, decide whether to enrich your sample or perform direct qPCR. The following chart compares the two options.

	Enrichment	**Direct qPCR**
Sensitivity	1 cell/sample	10 CFU/mL
Time	2 days*	2 Hours
Required Materials	FastOrange Enrichment Media	Centrifuge for 50 mL tubes

**Brettanomyces* takes longer. See table below.

Follow Step 1A for instructions on sample enrichment or Step 1B for direct PCR sample prep before continuing to Step 2.

A. Enriching Your Sample

For best results, we recommend FastOrange sample enrichment, which increases sensitivity and degrades dead cells. The enrichment protocols for the various kits using the FastOrange Enrichment Bottles are as follows. If using FastOrange Enrichment Broth, consult the Chai Knowledge Base Article "How do I use the 240 mL FastOrange Broth bottles for sample enrichment?" Always use sterile technique when transferring solution and vent containers during enrichment by twisting the cap so that the arrows are vertical to prevent pressure buildup.

Bacteria	
- ***Lactobacillus & Pediococcus* Real Beer Spoiler Test Kit** - ***Lactobacillus & Pediococcus* Real Beer Spoiler Identification Kit** - ***Lactobacillaceae* Test Kit** - **Acetic Acid Bacteria Test Kit**	Add 50 mL of sample to a 50 mL FastOrange B Enrichment Bottle and incubate it for 48 hours at room temperature. Leave the bottle standing vertically for lactic acid bacteria testing or flat on its side for acetic acid testing. Keep the bottle vented.
- ***L. acetotolerans* Test Kit** - ***Megasphaera* Test Kit** - ***Pectinatus* Test Kit**	Refer to Chai Knowledge Base Article "How do I enrich for anaerobic bacteria (*Megasphaera*, *Pectinatus*, *L. acetotolerans*)?"

Yeast	
- *S. diastaticus* Test Kit - Superattenuator Yeasts Test Kit (less sensitive Brett detection)	Add 40 mL of sample to a 40 mL FastOrange Wild Yeast Enrichment Bottle and incubate it for 48 hours at room temperature. Leave the bottle on its side and vent.
- Superattenuator Yeasts Test Kit (single cell Brett detection)	Perform separate sample enrichments in FastOrange Brett Broth for *Brettanomyces* and FastOrange Wild Yeast Broth for *S. Diastaticus*. Combine both resulting samples into a single 1.5 mL tube.
- *Brettanomyces* Test Kit	Add 40 mL of sample to a 40 mL FastOrange *Brett* Enrichment Bottle and incubate it for 72 hours at room temperature. Leave the bottle on its side and vent.

B. Concentrating Your Sample for Direct qPCR

If you choose to forgo sample enrichment, you must spin down a higher volume of sample using a 50 mL centrifuge before proceeding.

For clear beer samples:

1. Spin down 50 mL of beer in the 50 mL centrifuge for 15 minutes at max speed to create a pellet.
2. Decant most of the liquid, leaving approximately 1 mL remaining at the bottom of the tube. Vortex or pipette up and down 10 times to resuspend the pellet, then transfer the liquid into a new, sterile 1.5 mL tube.

For turbid, hazy beer samples:

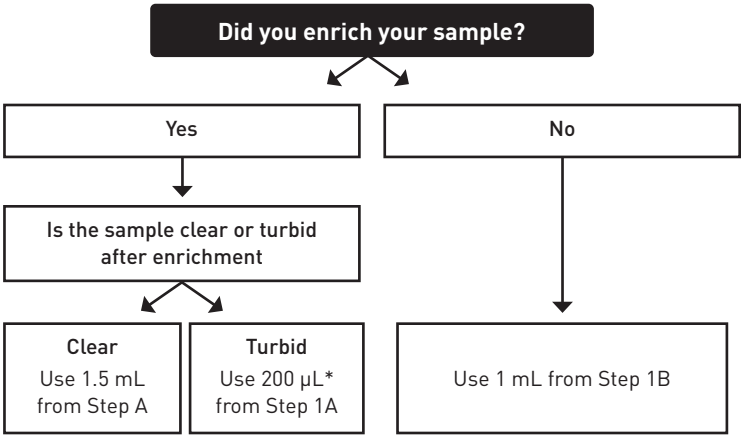
1. Spin down 15 mL of hazy beer sample in a 50 mL centrifuge tube for 10 minutes at max speed to create a pellet.
2. Decant some of the liquid, leaving approximately 5 mL remaining. Next, add 45 mL of distilled water and vortex or pipette up and down ten times to resuspend the pellet. Centrifuge the tube for another 10 minutes at max speed.

3. Decant most of the liquid, leaving approximately 1 mL remaining at the bottom of the tube. Vortex or pipette up and down to resuspend the pellet, then transfer the liquid into a new, sterile 1.5 mL tube

   Once you transfer your sample into the new 1.5 mL tube, proceed to Step 2.

II: Prepare Your Sample

A. **Follow the chart below to determine the volume of sample to pipette into a new 1.5 mL tube. Label the tube appropriately.**



*The range of acceptable starting sample volumes for turbid, enriched samples is 50 – 200 µL. Start off by using a 200 µl volume and reduce the pellet if necessary (described later under "What if my pellet is too large?"). For future runs, you'll learn what to expect for the pellet size of your various samples and can reduce the starting volume as needed.

B. **Place your 1.5 mL sample tube in a centrifuge and spin down for 3 minutes at a minimum of 5,000 g.**

   This corresponds to 10K RPM on most mini centrifuges. Make sure your centrifuge is balanced with another 1.5 mL tube with a similar volume on the opposite side of the rotor to avoid damage. The result should be a small pellet at the bottom of your tube.

   The width of the pellet should not exceed 2 millimeters.

Below are examples of pellet sizes you may encounter during your sample prep. The first three tubes have acceptable pellet sizes. The last two are too large.

Once you have an acceptable pellet size, pipette out as much of the supernatant (excess liquid) without disturbing the pellet.

What if I don't see a pellet?

In some cases such as with filtered beers, no visible pellet will form (see leftmost tube in above photo). Instead, assume that an "invisible pellet" has formed at the bottom of your tube and proceed with pipetting out the supernatant, leaving the bottommost 50 µL undisturbed at the bottom of your tube.

What if my pellet is too large?

Use a clean pipette tip to carefully pipette out some of the excess pellet. Spin down your sample for another 3 minutes at 10K RPM and reassess the pellet. Repeat as necessary until the pellet is an acceptable size. For future sample prep of the same beer type, you should spin down less sample volume to avoid needing to remove excess pellet.

C. **Wash your sample by adding 200 µL of Washing Buffer (yellow cap).**

Make sure to use a new pipette tip. Vortex the tube or pipette up and down ten times to resuspend the pellet in the solution. Spin down your tube for another 3 minutes at 10K RPM in a balanced centrifuge. Use a new pipette tip to remove as much of the supernatant without disturbing your reformed pellet. It is normal for the pellet size to decrease slightly in this step.

D. **Lyse your sample by adding 200 µL of Lysis Buffer (blue cap).**

Make sure to use a new pipette tip. Vortex the tube or pipette up and down ten times to resuspend the pellet in the solution. Next, incubate your tube in a dry bath at 80 ºC for 10 minutes. Your sample may undergo a color change after incubation.

Note: The Yeast and Mold Kit follows a slightly different protocol for the lysis step. Please see the PIKA 4e manual when performing the step for this kit.

After 10 minutes in the dry bath, spin down your sample in your centrifuge for another 3 minutes at 10K RPM. Now that your sample has been lysed, the sample DNA is in the supernatant of your tube rather than the pellet at the bottom of the tube. Pellets that form at this step contain remaining dead cells and debris. Transfer 100 µL of the liquid supernatant to a new, labelled 1.5 mL tube without disturbing the pellet and discard the old tube. This sample is now ready for PCR. You can save this sample for same-day PCR analysis by storing at 4 ºC.

III. Performing the PCR Run

A. **Label a new 1.5 mL tube as your Reconstitution Solution ("RS") and determine the required quantities of Master Mix (green cap) and Rehydration Buffer (white cap).**

Look at the tables on the back of the kit box to determine the amount of Master Mix and Rehydration Buffer you will need to prepare the Reconstitution Solution. The tables take the positive and negative control into account. Use the left table if running one kit and the right table if running two kits. If running two kits, the Reconstitution Solution from one kit can be used for both.

Vortex the Master Mix for 3 seconds or pipette up and down with a clean pipette tip, then pipette the required amount into your RS tube. Inadequate mixing of stock master mix may lead to improper amplification. With another clean pipette tip, pipette the required amount of Rehydration Buffer into the same RS tube. Once both the Master Mix and Rehydration Buffer are added, vortex your tube for 3 seconds or pipette up and down 10 times to mix, then spin down the tube in a balanced centrifuge for 20 seconds.

2 Kits per Run	Volume to pipette in µL		Total
Number of samples	Rehydration Buffer (White)	2X Master Mix (Green)	Volume in µL
2	66.0	99.0	165.0
3	77.0	115.5	192.5
4	88.0	132.0	220.0
5	99.0	148.5	247.5
6	110.0	165.0	275.0
7	121.0	181.5	302.5
8	132.0	198.0	330.0
9	143.0	214.5	357.5
10	154.0	231.0	385.0
11	165.0	247.5	412.5
12	176.0	264.0	440.0

If you ran 2 samples with the Brett Kit and 3 samples with the LP kit, you'd follow the 2 Kit table (Number of samples is 2 + 3 = 5) and pipette 99.0 µL of Rehydration Buffer 148.5 µL of Master Mix into your tube.

1 Kit per Run	Volume to pipette in µL		Total
Number of samples	Rehydration Buffer (White)	2X Master Mix (Green)	Volume in µL
1	33.0	49.5	82.5
2	44.0	66.0	110.0
3	55.0	82.5	137.5
4	66.0	99.0	165.0
5	77.0	115.5	192.5
6	88.0	132.0	220.0
7	99.0	148.5	247.5
8	110.0	165.0	275.0
9	121.0	181.5	302.5
10	132.0	198.0	330.0
11	143.0	214.5	357.5
12	154.0	231.0	385.0
13	165.0	247.5	412.5
14	176.0	264.0	440.0

If you ran 1 sample with the Diastaticus kit, you'd follow the 1 Kit table (Number of samples = 1) and pipette 33.0 µL of Rehydration Buffer and 49.5 µL of Master Mix into your tube.

B. **Prepare your PCR tube and cap strips by cutting down the number of tubes and caps you need with a sterile razor or scissors.**

Keep the plastic film on the tubes and protect them from light when not in use. Note that you'll need tubes for your positive and negative control for every kit that you run. For example, if you're running two samples on one kit, you'll need a strip with four tubes total: one for the positive control, one for the negative control, and two for your samples. If you're running two kits, you'll need a strip from each kit.

C. **Pipette 25 µL of Reconstitution Solution into each tube.**

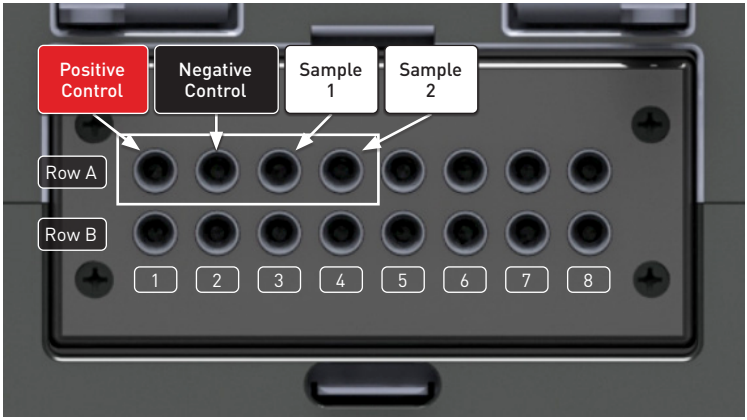
Carefully remove the plastic film to avoid disrupting the lyophilized material at the bottom of the tubes. Use a new pipette tip and pipette the liquid in at an angle to avoid inserting your pipette to the bottom of the tube.

D. **Pipette 5 µL of your Positive Control (Spoiler DNA: red cap) into the first tube, 5 µL of your Negative Control (Rehydration Buffer: white cap) into the second tube, and 5 µL of each sample into the subsequent tubes.**

Use a new pipette tip for each tube and avoid cross contamination. There should be a final volume of 30 µL of solution in each tube. Mark one side of your strip with a dot using a permanent marker to keep track of which side contains your Positive Control. Avoid marking the top or side of any of your tubes, as this may affect the optical readout during the run and lead to inaccurate results.

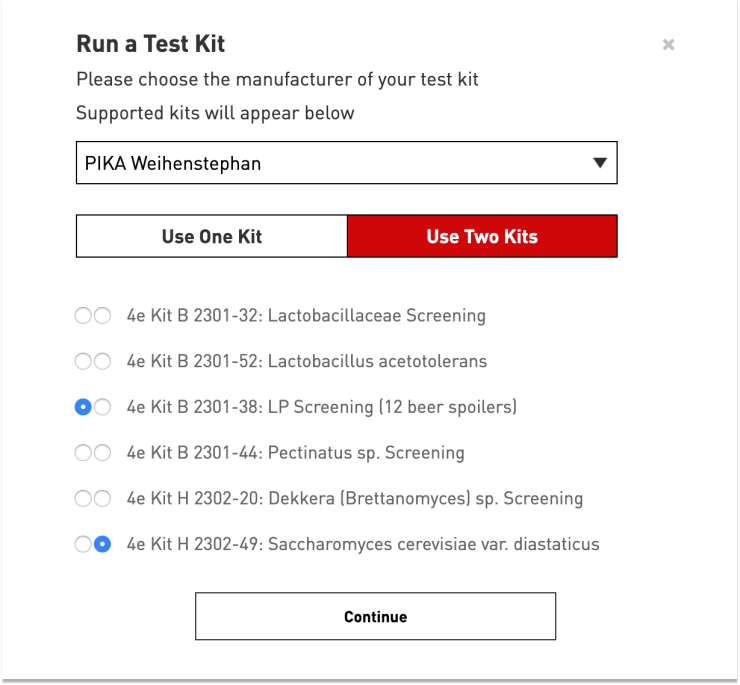
E. **Cap your tubes, spin them down in a balanced centrifuge, and place them in your Open qPCR.**

Your positive control (marked with a dot on one side of your strip) should start in Well A1. If you're running two kits, your second positive control will start in Well B1.



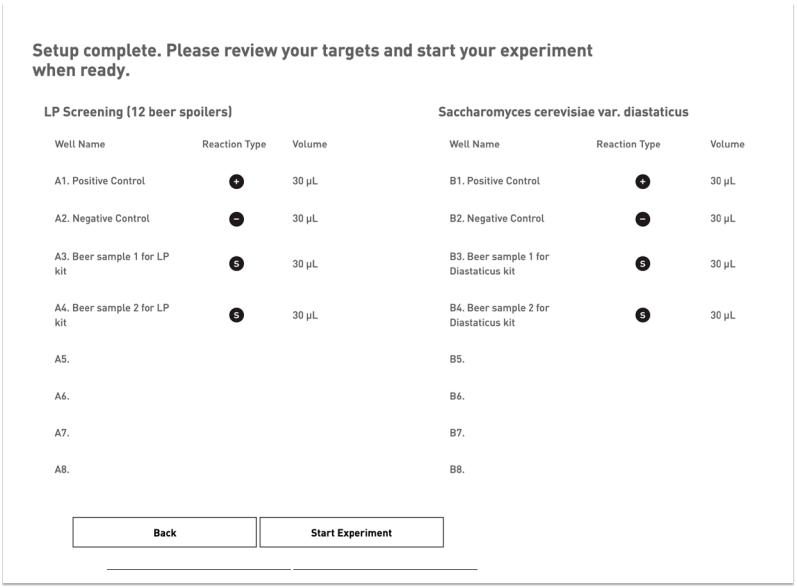
F. **Set up your experiment using the Open qPCR software.**

From the Open qPCR home screen, click "Run a Test Kit," select PIKA Weihenstephan as the manufacturer, and choose "Use One Kit" or "Use Two Kits" depending on what you need. Select which kit(s) you are using. If using two kits, note which kit you assigned first and second. Input the names of your samples into the appropriate wells, with the samples and controls for your first selected kit occupying the A wells and the samples and controls for your second selected kit occupying the B wells. Leave unoccupied wells blank.



G. **Review your targets and select "Start Experiment" to begin the run.**

Once you start the run, the screen will show that the machine is heating up, then display a timer countdown. The run will take 75 minutes to complete. You can close your browser or disconnect your computer during this time without interrupting the run. You can navigate back to the experiment from the home screen. Once the run finishes, dispose of the tube strips in your regular trash. Do not open the strips once the run completes as doing so may contaminate your environment with PCR amplicons.



IV: Reading Results

Once the run completes, navigate to the experiment and read the results for each well.

For a successful experiment, the following criteria must be met:

- Well A1 (Positive Control) should generate a C_q value and indicate "Valid" under Results.
- Well A2 (Negative Control) should not generate a C_q value and indicate "Valid" under Results.

If both your Positive and Negative Control are valid, your sample wells will read Positive or Negative under Results and indicate "Low," "Medium," "High," or "Not Detectable" depending on whether or not the presence of the target was detected.

If Well A1 and/or Well A2 generate "Invalid" results, your samples will generate "Unknown" results and you will need to repeat the experiment to validate them.

Below is an example of a successful run. In this case, the sample in in Well A3 tested positive for LP, while the sample in Well A4 tested negative for LP.

Experiment:

A.LP Screening (12 beer spoilers)

LP Screening (12 beer spoilers)

Well	Cq	Results	Amount	Notes
A1. Positive Control	28.25	Valid	—	
A2. Negative Control		Valid	—	
A3. Sample 1 - turbid, enriched	35.95	Positive	Low	
A4. Sample 2 - clear, direct qPCR		Negative	Not Detectable	

If your controls are valid but your sample result does not indicate Positive or Negative, your sample likely experienced PCR inhibition.

Inhibition can happen from a variety of factors. Below are some preventive steps to achieve better results:

- Perform a sample enrichment prior to running the kit
- Ensure that your pellet does not exceed 2 mm in diameter (large pellets often inhibit PCR)
- Remove as much of the supernatant as possible without disturbing the pellet after the first sample spindown and wash steps
- Perform an additional washing step: i.e, after one 200 µL wash, perform another 200 µL wash with your sample
- Use a higher volume of washing buffer during the washing step(s): i.e, instead of a 200 µL wash, perform a 400 µL wash

Viewing Full Results

The Open qPCR software provides more detail for your kit runs by generating an Amplification Curve. To view the Amplification Curves for your experiment, select "View Full Results" at the bottom of your results screen. You can find more information on reading Amplification Curves on the Chai Knowledge Base.

Exporting Kit Runs

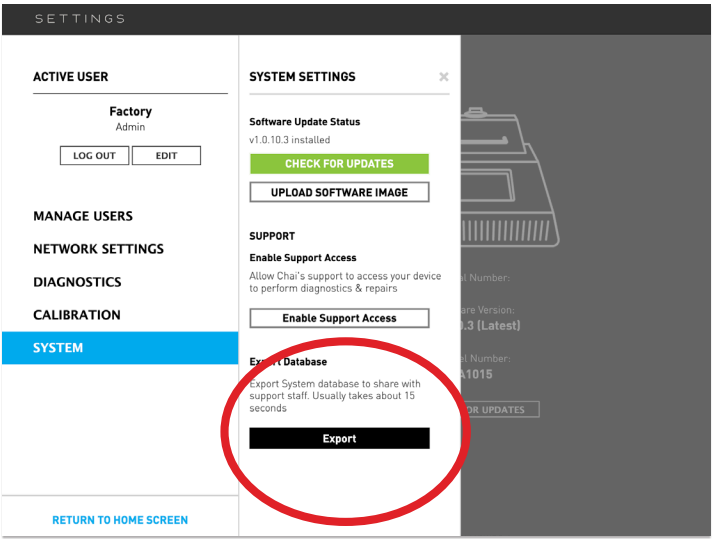
Although not typically necessary for beer spoiler detection, it is possible to export the results of your individual kit runs. You can do this by selecting the icon at the top left of the Open qPCR software and selecting the "Export" option that comes up in the side window.

This will generate a zip file containing .csv files of C_q values, Amplification Curve data points, and Temperature Logs for your experiment. Note that this process is specific to the individual kit run and is not the same thing as exporting your database. Graphics of the results table and curves on the "View Full Results" page can be saved by taking screenshots.

V. Further Questions?

More detailed articles related to enrichment, test kits, and post-run analysis can be found under "Beer Spoilage" on our Knowledge Base at support.chaibio.com.

If you have other questions regarding the PIKA 4e protocol, we'd be happy to answer them at support@chaibio.com. For inquiries about your results, please export your database and email it to us with your questions. You can export your database by navigating to **Settings >> Systems >> Export** under "**Export Database**" in the software.



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