- INSTRUCTION MANUAL -

PSILOCYBIN QTEST BULK REAGENTS
(FOR USE WITH QTEST PHOTOMETER)

Precise analysis of the psilocybin concentration in mushrooms, truffles and extracts

IMPORTANT INFORMATION - READ THIS FIRST!

- Wear protective clothing, gloves, and glasses, and always remember the detection solution contains an acid! Particular care must be taken when pipetting the solution into the cuvettes.
- Use this kit at room temperature (if stored in a refrigerator, let it warm up for one hour first).
- Evaluate the result immediately after the 15-minute development time.
- This kit only works with dried fungal material (solid or powdery substances). It does not work with fresh mushrooms.
- Cautious handling of the semi-blunt needle, and hot water (**risk of injury/burn**).
- The cuvettes have a small triangle that must point in the direction of the LED light beam.
- Read all the instructions (this one and from the QTest photometer) at least once before beginning the test.

INSTRUCTIONS FOR USE

1. FIELD OF USE

- This test kit is for the experienced user who is aware of the dangers of pipetting acids. To increase the accuracy of the result, we recommend using a 1 ml pipette with ejectable tips, but disposable syringes are also included.
- The Psilocybin QTest totals the amount of both active ingredients (psilocybin and psilocin) in your fungal sample. Although we present the total in terms of psilocybin — only because this is the most well-known alkaloid — the results give you the potency of your mushrooms, including both psilocybin and psilocin.
- With the reagents in this test kit you can perform at least 25 concentration analyses, and there is enough reagent to perform an additional 5 blank measurements.

2. PREPARING FOR THE TEST

Take out all the items from your kit. Along with these instructions, you should have:

- 4 x Protective gloves
- 1 x Big brown plastic vial with a screw cap (40 ml clear extraction solution -> **have to be stored cool!**)
- 1 x Vial with the detection solution (30 ml detection solution - slightly yellow)
- 5 x 1 ml Syringe
- 5 x Semi blunt needle
- 25 x Syringe filter tip (Sterifilt)
- 25 x 1.5 ml Reaction tube
- 25 x 2 ml Reaction tube
- 25 x Clear cuvettes

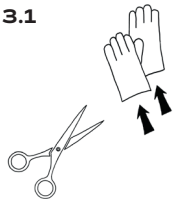
You will also need a milligram scale, a water bath at 176 °F or 80 °C (or a pot to boil water), tweezers (or kitchen tongs), scissors/grinder and either a small funnel or a creased piece of paper to pour the mushroom material into the reaction tube with extraction solution vial carefully.

When testing dried material: Before you begin the test, you must thoroughly homogenize your dried mushroom material. A mortar and pestle for freeze-dried samples, a typical grinder, or even scissors with a 50 ml Falcon tube works best. You don't need to powder the sample, as this will result in clogging the filter much more easily. Then mix the chopped material thoroughly. You will use 50 milligrams (mg) of material for the test. The result will tell you the percentage of psilocybin in 1 gram (g) of the remaining homogenized material (in terms of mg/g).

When testing extracts: Extracts are usually more concentrated. To ensure that the color result of the test still fits within the linear range of the Psilocybin QTest, you have to reduce the amount of starting material accordingly. Before testing homogenize your substance. Typically, methanol extracts are triple-concentrated, so if you have a crude extract, we recommend dividing the amount you use for the test by factor three (~16,7 mg), or diluting your extract in extraction solution and using just a portion. If the extract is considerably more concentrated, reduce the initial quantity accordingly. The extract must be as solvent-free as possible, as this can interfere with the extraction solution. Remember that you have to multiply the result from the photometer in the end by the factor used to obtain the actual result for the remaining sample.

3. PERFORMING THE TEST

3.1



First, wear the enclosed nitrile gloves and wear appropriate protective clothing and safety glasses. If you wear contact lenses, remove them. The vials' liquids contain acids that may cause skin irritation or severe eye damage. If you come in contact with the liquid, remove the affected clothing immediately and rinse the affected skin area with water for several minutes. In case of eye contact, rinse the affected eye with running water for several minutes. (Keep pets, especially cats, away from the testing area.)

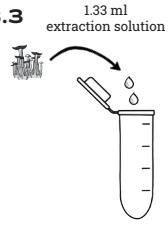
3.2

Carefully weigh **50 mg (0.05 g)** of your homogenized material using an accurate milligram scale. For concentrated extracts, use a proportionally lower amount.

Note: *Check out our store: We have an affordable, accurate, and calibrated scale available that you can trust.*

Preheat your water bath to 176 °F (80 °C) or around ~ 1 liter of water (about four cups) in a pot on the stove or a kettle. When the water boils, turn off the stove.

3.3



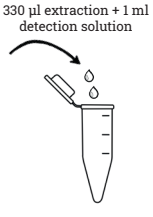
Open the 2 ml plastic reaction tube and pipet 1.33 ml extraction solution into it (if you are using the syringe, you have to do it in two steps first 1 ml and then 0.33 ml). Then carefully pour in your mushroom material using a small funnel or creased piece of paper. Put the lid back on tightly and shake the reaction tube for approximately 30 seconds. Then, put the vial down and wait for 10 minutes. Repeat the shaking process twice during this waiting period, once at about 3 minutes and again at around 7 minutes. If the material sticks on the top, shake it more often while waiting.

3.4

If you have a centrifuge, you can also centrifuge off the biomass and use the particle-free supernatant. If not then unpack the syringe and the syringe filter tip. Attach the filter tip closely to the syringe. Open your plastic reaction tube and insert the filter tip (make sure to insert the round filter as straight and tight as possible into the 2 ml tube!). The filter should be able to be pushed just a little way into the reaction vessel and seal it completely. Now you can turn the reaction vessel upside down, pull the syringe plunger completely, and hold it there. This will create negative pressure, and the syringe will slowly fill with liquid, you will just need 0.33 ml for the next step. But we recommend drawing up about 0.5 ml (if the filter clogs, you can clean it carefully and try again).

Remove the syringe from the plastic tube. Also, detach the sterile filter from the syringe and gently tap the syringe with your fingernail while holding it upright. In this way, any air bubbles will rise and you can carefully push out as much extraction solution until exactly 0.33 ml remains in the syringe without air bubbles. The most accurate way to do this is to use a pipette, if you don't have one then follow our instructions as closely as possible.

3.5



Pipet 0.33 ml (330 µl) of the filtered extraction solution into the smaller 1.5 ml reaction tube using a pipet for more accuracy or try to be as accurate as possible with the syringe. Add 1 ml of the detection solution and close the reaction tube. Mix the liquids by inverting the tube several times.

NOTE: *The liquid in the reaction tube may now be greenish. This color change indicates the presence of psilocin in the sample but does not provide a quantitative result. You still need to incubate the tube in hot water.*

PREPARE A CONTROL BLANK: *Scientifically correct at this point would always be to additionally prepare a reaction tube following the same steps as for the sample tube, but without adding any sample.Use only the extraction and detection solutions, following the same protocol, and incubate this tube in the water bath as well.*
This tube serves as a blank — a reference without sample — that you can later use in the photometer to correct for any background color or signal.

3.6

Incubate the samples at 176 °F (80 °C) for 15 minutes in a water bath or a pot/kettle. The reaction tubes should be quite full and therefore swimming upright on top of the water, if you have you can use a floating rack.

Note: *To heat up some water, remove the pot from the heat source, turn off the stove, and wait for 5 minutes. If using a pot/kettle ensure the water is* ***no longer boiling****. Too high temperatures lead to an increase in the measurement results and should be strictly avoided.*

3.7

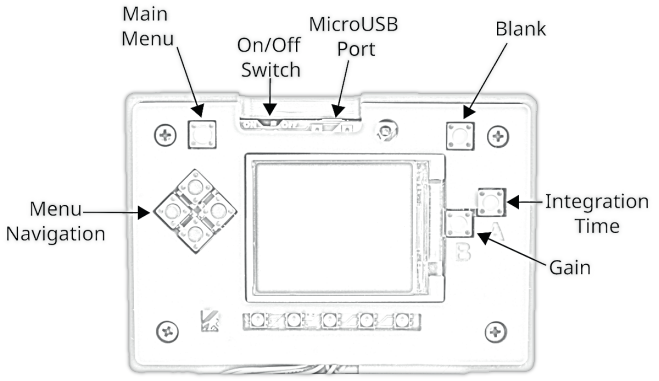
The solution in the tube will start to change color. After 15 minutes, carefully remove the floating tube from the water bath. **After a few minutes, the tubes will be cool enough to handle.** In the meantime start your photometer, select the psilocybin detection method, and let the LED equilibrate for at least 5 minutes. Now you can open the reaction tube and pipet the whole solution into one cuvette. Begin by blanking the photometer using your prepared blank, and then proceed with measuring your sample.

3.8

RESULTS: The corresponding concentration of psilocybin/psilocin is listed as milligrams per gram of material (mg/g). This concentration applies to your remaining homogenized material.

For extracts, you need to multiply the number you read on the photometer by the same factor that you reduced your starting material at the beginning of the process. For example, if you tested a 3X extract and reduced your starting material by a factor of three, you must multiply by three to obtain the correct result. Similarly, if you tested a 10X extract (using 5 mg), you must multiply by ten.

4. SHORT INSTRUCTIONS QTEST PHOTOMETER



First Steps:

- Switch on the photometer.
- Read the "README" instructions that you can find at the photometer section in our webshop (connect it to the computer).
- The intro splash screen appears followed by the default absorbance screen.
- Press the top left button to enter the menu and use the cluster of 4 buttons on the left- hand side to navigate "↑↓" the menu.
- To select a mode (e.g., transmission) press the right "→" button in the cluster.
- Press the top left button to go back to the menu at any time.
- Select the psilocybin measurement using the menu navigation.
- Let the LEDs warm up!
- Before starting a measurement, you need to blank (aka "zero" or "calibrate") the colorimeter. This sets the measurement background against which your sample is compared. Place the blank sample in the cuvette holder. Place the black light- blocking cover over the cuvette holder (the face marked triangle pointing downwards should be positioned parallel to the photometer screen, and ensure that it is pointing toward you).
- Press the top right Blank button. You should see a flashing "Blanking" line on the screen.
- Once the blanking has finished, place your sample in and record the concentration value.
- Now several samples can be measured one after another without needing to press a new button.
- Do not allow acid to drip onto the photometer, especially the optics. Work cleanly!
- Please follow the same usage and safety instructions as outlined in the photometer manual.

SIGNAL CHAT AND FREQUENTLY ASKED QUESTIONS

Check our FAQ Section at our https://www.miraculix-lab.de/en/qtest-photometer/step-by-step-guide

For assistance in evaluation, especially with the photometer, join our Photometer Signal Chat (quick Q&A with us and other photometer users!).

5. LIMITATIONS

- The Psilocybin QTest was developed to enable the most linear and accurate concentration determination of psilocybin/psilocin. Even norbaeocystin and baeocystin do not show false positives. As the test is also incredibly sensitive, weak false positive reactions can occur with fungal material that does not contain psilocybin. The cross-reaction is most likely a false positive reaction to reactive indole. So when testing wild mushrooms, correlate your possibly weak concentration result with the morphology of your mushrooms and make sure they are psilocybin-containing mushrooms.
- Mushrooms collected in the wild can always be so-called doubles. This is especially dangerous for the poisonous *Galerina marginata*. A positive concentration result does not exclude the possibility that the homogenized quantity also contains other, potentially deadly poisonous mushrooms.
- Sources of potential inaccuracies in concentration results: samples were boiled under additional heat and not just incubated in hot water will result in a higher concentration result (nearly black), inaccurate weighing (scale not accurate), variations in the amount of extraction liquid added, expired/discolored detection reagents, not all Psilocybin/Psilocin was extracted due to short extraction time, too short or too long incubation time, non-representative sample tested (homogenized quantity too small or insufficiently homogenized).

6. STORAGE AND SHELF LIFE

Store the reagents in the refrigerator (35 - 46 °F or 2 - 8 °C) and out of direct sunlight for a shelf life of at least 18 months after production. Especially the extraction solution should be stored always cooled. Taking aliquots of the extraction solution directly in the tubes increases stability, because the whole solution does not have to be taken out of the refrigerator several times The extraction liquid may turn slightly yellow over time. That's okay. It is still usable.

7. WARNINGS

- Pay attention to the labels on the reagent vials.
- The test kit must always be kept away from children and animals.
- When using the test kit, suitable gloves, protective clothing, and face/eye protection must be worn.
- If you wear contact lenses, remove them before use.
- All delivered materials and liquids should not be ingested or inhaled.
- The included liquids in the test kit contain various acids that can cause skin irritation or severe eye damage/irritation. Skin, eyes, mouth, or clothing contact should be avoided.
- If contact occurs with the above-mentioned areas, remove the relevant clothing immediately. Rinse or flush affected skin areas immediately with plenty of water for several minutes.
- In case of contact with the eyes, rinse them immediately with plenty of water for several minutes.
- Cautious handling of the semi-blunt needle and hot water (**risk of injury/burn**).

8. DISCLAIMERS

- Miraculix QTests are to be used only for research purposes and to quantify unknown substances.
- Your sample could be adulterated with one or more unknown substances that cross-react with the reagents, affecting the quantitative result.
- The result does not mean your sample is safe to consume. It could still contain unwanted substances and impurities that can be harmful or even deadly.
- The result does not serve as an identification for the mushroom species. Psilocybin mushrooms can have a similar appearance to poisonous mushrooms. Mushrooms that do not contain psilocybin could cross-react with the reagents and produce minor stainings. This is a false positive reaction presumably to reactive indole.
- Miraculix assumes no responsibility for using or misusing the test kit or the results.
- Samples processed with the test kit are not to be consumed.
- Patent pending technology.