

1 Heat Block Set-up

1. Preheat the heat block at 37–44 °C (see table below). Heat to 47 °C for solid and semi-solid fats and tallows.

Heat Block Dial Settings						
Dial Settings:	1	2	3	4	5	6
Temp (°C):	10	20	30	40	50	60

2 Preparing Reagent

1. Remove the Preparation Reagent from the refrigerator and allow it to stand at room temperature (18–25 °C) for 1–2 hours.
2. Before attaching the dispenser, gently swirl the contents of the bottle.
3. Attach the Preparation Reagent dispenser to the bottle. Volume on the dispenser can be set as needed.
4. To eliminate air bubbles and oxidized reagent, dispense 4–5 aliquots of the reagent into a waste container immediately before use.

3 Preparing Samples

1. See the table on the back of this card and follow the instructions for the type of sample you are testing.
2. Cap conical tubes and vortex the samples at the fastest dial setting for 1 minute unless stated otherwise on the back of this card (e.g. fresh organ samples).
3. Place prepared samples in the heat block set at 37–44 °C or 47 °C for solid and semi-solid fats and tallows, for 10 minutes.
4. Vortex at the fastest dial setting for 15 seconds.
5. Place the test tubes in the heat block at 37–44 °C for another 5 minutes.

4 Preparing Filtration Unit

1. While samples are being heated, label a new glass test tube for each sample and place the new tubes in the acrylic base of the SafTest™ filtration unit.
2. Position the membrane holder (with membranes attached) onto the acrylic base.

5 Filtering Samples

1. Remove samples from the heat block.
2. Pour the sample solutions into the membranes, filling each membrane to the top. Cap membranes to seal them.
3. Turn on the filtration unit and slowly increase the vacuum pressure to 5–10 in.Hg.
4. Filter until the solution is homogenous and clear. Samples with high fat content may appear slightly cloudy and may need further dilution to completely solubilize the fat.

NOTE: *Improper filtration or too high of a fat content is indicated by the formation of two layers. Should this occur, dilute, reprepare, and refilter the samples again using new membranes.*

5. When filtering is complete, reduce the pressure to 0 and turn off the filtration unit.
6. Discard the membrane holder when all membranes have been used. Do not reuse membranes.
7. Remove the test tubes containing the filtered samples. Cap the tubes and place them in the heat block to keep warm until you perform the test.
8. Continue with **Step 5: Prepare Samples** on kit protocol.

Sample Type	Preparation Instructions
Ground meats, meals, pumpable meats and digests	<ul style="list-style-type: none"> • Weigh 1.0 gram of sample (± 0.05 g) into the bottom of each conical tube. Pack loosely. • Add 10 glass beads to each conical tube. • Set the Preparation Reagent dispenser to 3.0 mL and dispense 1 aliquot into each conical tube. • This is a 1:4 dilution
Fish and fish steaks	<ul style="list-style-type: none"> • Chop into 0.5 cm cubes. • Weigh 1.0 gram of sample (± 0.05 g) into the bottom of each conical tube. Pack loosely. • Add 10 glass beads to each conical tube. • Set the Preparation Reagent dispenser to 3.0 mL and dispense 1 aliquot into each conical tube. • This is a 1:4 dilution
Fresh organs	<ul style="list-style-type: none"> • Chop into 0.5 cm cubes. • Weigh 1.0 gram of sample (± 0.05 g) into the bottom of each conical tube. Pack loosely. • Add 10 glass beads to each conical tube. • Set the Preparation Reagent dispenser to 1.5 mL and dispense 1 aliquot into each conical tube. • Vortex at fastest dial setting for 1 minute. • Dispense an additional 1.5 mL of Preparation Reagent. • Vortex at fastest dial setting for 1 minute. • Final dilution is 1:4.
Finished products: wet	<ul style="list-style-type: none"> • Mix sample in a blender until it is a homogenous solution. • Weigh 1.0 gram of sample (± 0.05 g) into the bottom of each conical tube. Pack loosely. • Add 10 glass beads to each conical tube. • Set the Preparation Reagent dispenser to 3.0 mL and dispense 1 aliquot into each conical tube. • This is a 1:4 dilution
Finished products: dry	<ul style="list-style-type: none"> • Using a coffee grinder, grind the sample into a powder. • Weigh 1.0 gram of sample (± 0.05 g) into the bottom of each conical tube. Pack loosely. • Add 10 glass beads to each conical tube. • Set the Preparation Reagent dispenser to 3.0 mL and dispense 1 aliquot into each conical tube. • This is a 1:4 dilution
Tallows and greases	<ul style="list-style-type: none"> • Weigh 0.20 gram of sample (± 0.05 g) OR dispense 200 μL of sample using a positive displacement pipette into the bottom of each conical tube. • Set the Preparation Reagent dispenser to 1.8 mL and dispense 1 aliquot into each conical tube. • This is a 1:10 dilution <p>NOTE: Step 3 on the front of this card completes the sample preparation for tallows and greases, which do NOT require filtering. After heating, remove the conical tubes from the heat block. Then proceed to the Analyzing Samples step on kit protocol.</p>
Oil	<ul style="list-style-type: none"> • Dispense 500 μL of sample and into the bottom of each conical tube. • Add 4.5 mL of Standard Prep Reagent. • This makes a 1:10 initial dilution. • Vortex the sample for 1 minute and warm in heat block at 37–44°C for 10 minutes. • Vortex the sample for another 15 seconds and place the sample back in the heat block. The test will be performed using the 1:10 dilution. Additional dilutions may be needed later. Keep samples in the heat block until all the tests are completed.