# **EpiShear™ Cooled Sonication Platform**

(version C1)

Catalog Nos. 53080 (1.5 ml), 53081 (15 ml) & 53082 (50 ml)

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#### Overview

Active Motif's EpiShear™ Cooled Sonication Platform was designed to improve sample-to-sample reproducibility when using a probe sonicator to shear chromatin or DNA by enabling the tip of the probe to be positioned at the same depth in the sample each sonication. The platform has been machined out of stainless steel and aluminum, and includes a hand crank, a height counter and a vertical alignment tool, so once you have determined the optimal settings for your probe, sample volume and tube type, you can recreate your sonication conditions over and over.

Included with each platform is your choice of a Tube Cooler that will hold 1.5-2.0 ml microfuge, 15 ml or 50 ml tubes. The Tube Coolers keep the sample cold during sonication, so you don't need move the sample to and from an ice bucket during sonication. With a programmable sonicator like Active Motif's EpiShear Probe Sonicator (Catalog No. 53051), you can simply set the duration of the On and Off pulses, place the sample in the cooler, press Start, then walk away. The cooler can keep the sample cold for up to 2 hours of sonication. Each cooler is also sold separately.

The platform is supplied pre-assembled inside a sound enclosure. Also included are an alignment tool and hex wrenches that enable the platform height to be adjusted to your ideal settings.



format	catalog no.
1 platform	53080
1 platform	53081
1 platform	53082
1 cooler	53076
1 cooler	53077
1 cooler	53078
110 V 230 V	53051 53052
	1 platform 1 platform 1 platform 1 cooler 1 cooler 1 cooler

As this manual contains many images, we recommend that you download its PDF version from our website; the pictures will then be in color, and you will be able to zoom in for better detail.

## **Included Components**

The Cooled Sonication Platform is supplied with the following components.

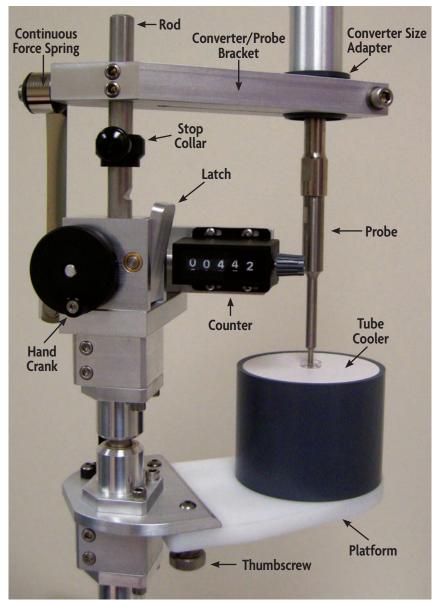
- Sound enclosure with measurements of 28" H (with chimney) x 14.25" W x 12" D (711 mm x  $362 \text{ mm} \times 305 \text{ mm}$ )
- Probe chimney
- Sonication platform
- Choice of a 1.5 ml, 15 ml or 50 ml Tube Cooler
- · Alignment tool
- · 3 hex wrenches
- Alternate spring bracket with set screw (optional; see below)

#### Alternate spring bracket

The alternate spring bracket (shown below) is not normally needed. It was supplied in case you need to remove the converter/probe bracket from the top of the rod. You would need to do this only if your converter/probe is too large to be held by the bracket. See Appendix – Section A for instructions on how to remove the converter/probe bracket and replace it with the alternate spring bracket.



### **Parts Diagram**



The continuous force spring helps support the weight of the platform and enables it to move up and down the rod smoothly, either using the latch or the hand crank. The stop collar is used to set the highest point on the rod that the platform can be set. The two thumbscrews underneath the platform enable you to change the platform position both forward/backward and left/right so that the probe can be centered in the tube.

### **EpiShear™ Cooled Sonication Platform**

During shipment of the EpiShear Cooled Sonication platform, the probe chimney, tube cooler, alignment tool and hex wrenches are placed inside of the sound enclosure. Carefully remove each item and perform a visual inspection to detect any damage that may have occurred during shipment. Before disposing of any packaging material, check carefully for small items.



# A. Attachment of the Chimney

The chimney that will house the probe sonicator is shipped inside the sound enclosure and must be secured to the top of the sound enclosure prior to use. To attach the chimney, first remove the three bolts from the screws on the top of the sound enclosure. Then, align the chimney onto the screws with the chimney protruding from the unit. Finally, tighten the bolts onto the screws.







### B. Placement of the Converter/Probe

To ensure sample-to-sample consistency, the platform has a bracket secured near the top of the rod that holds the sonicator converter/probe. This bracket enables you to fix the placement of the probe tip. Depending on the probe to be used, you may need to adjust where the converter bracket is located vertically on the rod. Typically, the black Stop Collar is placed on the top gear of the rod, and the bracket is secured just above the collar (right). (The Stop Collar is used to set how far up the rod that the platform can be raised. Remove the pin so that you can slide the collar to its desired height, then reinsert the pin to fix the collar at the chosen rod gear.)

Once you have determined where the bracket will be placed, use the supplied hex wrench to loosen the set screws. Adjust the bracket to its new height and use the hex wrench to tighten the set screws so that the bracket will not move on the rod.

You will also need to determine at what height in the bracket the converter/probe will sit. It is suggested that you place tape or some kind of marking on the converter (right). This will enable you to return it to the exact same position in the bracket in the event somebody moves (or removes) the converter/probe from the bracket. Once the converter/probe is placed into the bracket, secure by tightening the screw to the converter size adapter. **DO NOT OVERTIGHTEN**. The bracket should firmly secure the converter/probe to the platform, but care must be taken not to damage the converter.

Note:

Depending on the size of your converter/probe, you may want to remove the converter/probe bracket and replace it with the alternate spring bracket. (See Appendix Section A)







#### C. Calibration of the Platform Counter

The counter on the platform needs to be set to an appropriate "zero" point to enable you to return the platform to the same height consistently once you have determined the appropriate height for your purposes. We suggest that the "zero" point be set at the bottom of the drive screw, meaning the lowest point to which the platform can be cranked.

To set the platform to the bottom of the drive screw, turn the crank clockwise until the platform base no longer continues downward; then, turn the dial on the analog counter clockwise to "0000" (right). From this point on, a one unit increase in the counter equals a 0.1 mm increase in the height of the platform.

Note:

If the platform goes too low, it may not move upward when you begin to turn the crank. Gently lift underneath the platform as you crank to engage the gears completely.



# D. Alignment of the Platform Stand

The platform stand can be adjusted in both the horizontal (x-y) and vertical (z) planes, ensuring that the probe is centered in the tube and that its tip is at the chosen depth in the sample. The provided Alignment Tool is used to set an approximate alignment for the tube and probe. It is included because when a tube is placed in a Tube Cooler, you cannot see the probe tip or its position relative to the bottom of the tube.

The Alignment Tool is used to make initial settings, which are refined using a tube in the Tube Cooler. (This prevents possible damage to the probe that could occur if you simply raised the platform without knowing how deep in the tube the probe will go.)

You should have already placed your converter/probe in the bracket and set the counter to "zero", as described above. To begin, lower the platform and place the Alignment Tool in the hole on the stand. To lower the platform, simply press in the latch located between the counter and the crank (right). After placing the Alignment Tool in the stand, move the entire platform up to the top of the rod, against the Stop Collar.

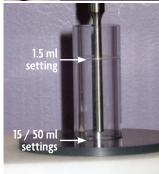


The crank is then turned to slowly raise the stand up the rod. When the probe begins to enter the Alignment Tool, you may need to adjust the position of the stand so that the probe is centered in the tool. Simply loosen the two thumb screws underneath the stand, use one hand to move the stand forward/backward and left/right until the probe is centered, then re-tighten the thumbscrews. You may need to refine the position again slightly when you test with a tube in the Tube Cooler (right).

Continue cranking the stand up in order to set the probe to its chosen depth. The line that is etched into the Alignment Tool is the approximate depth of a 1.5-2.0 ml tube; the bottom of the Alignment Tool is the approximate depth of both a 15 ml and a 50 ml tube.

Gently crank until the tip of the probe is either even with the etched line (if you will be sonicating in microcentrifuge tubes) or until it gently touches the bottom of the Alignment Tool (if you will be sonicating in 15 ml or 50 ml tubes, as shown to the right).





The platform is now set so that the probe will touch the bottom of the tube, which is not desired. You must adjust the platform slightly so the probe does not touch the tube during sonication. Use the crank to slightly lower the platform so the probe tip is the distance from the bottom of the tube desired for your experiments. This level will depend on the volume of the samples that will be sonicated, so you must set the final depth based on the sonication protocol that will be used.

After the probe has been positioned to the desired depth, the settings should be refined using a tube in the Tube Cooler. Because of slight variations in the shape and thickness of tubes from various manufacturers, the Alignment Tool may not provide you with the exact settings that will work with your tubes.

To refine the settings, press in the latch, lower the platform, remove the Alignment Tool, place a tube that will be used for sonication in the Tube Cooler (being certain it is all the way in the cooler), then place the cooler in the stand. Raise the platform back up until it is against the Stop Collar. You may now need to use the thumbscrews again to slightly re-adjust the stand so the probe is centered exactly in the tube. Lift the tube up and move it around slightly to verify that the probe tip is at the desired height from the bottom of the tube. Adjust the stand height if needed.

It is important that you now write down the number on the counter. This setting will be used for all experiments. While all you need to do to set the probe at this position is to move the platform up and down, if another user raises or lowers the platform using the crank, or if they change the counter, your positioning will be lost. By recording the counter value you can replicate your desired settings. Simply re-zero the platform as before, then crank the platform up to your setting.

### Using the Cooled Sonication Platform

#### **Benchtop Tube Coolers**

The Benchtop Tube Coolers can keep the temperature of a sample at or below  $4^{\circ}$ C for up to 2 hours of sonication if the cooler has been placed in a -20°C freezer overnight. If a large amount of time will pass between each sonication, return the cooler be to a -20°C freezer between samples. **Do not** place coolers at -80°C for more than 30 minutes as they may be damaged.

Note:

If the Tube Cooler was kept in a -20°C freezer overnight, it will be sufficiently cold to freeze your samples in about 5 minutes, unless you begin sonication. Therefore, to prevent your sample from freezing, do not place it in the cooler until you are ready to begin sonication. Keep your sample on ice and the cooler in the freezer until all preparations for sonication have been completed.



#### Sonication

Ensure that the converter/probe is positioned properly in the converter/probe bracket and that the platform settings are those that you determined to be appropriate for your tube type and sample volume. If need be, reposition the converter/probe to the mark you placed on it and re-zero the platform, then re-set it to its desired position.

Turn on your probe sonicator and adjust its settings to your preference. Press the latch and move the platform down the rod. Place an open tube containing your sample in the Tube Cooler, then place the cooler in the platform stand. Raise the platform until it is against the Stop Collar, and ensure that the appropriate counter setting has been reached. Lift the tube up and move it around slightly to verify that all is as expected. After verifying this, ensure that the tube is pressed down all the way in the cooler. You are now ready to begin sonication following your protocol.

After sonication is complete, press the latch and lower the platform so that you can remove the sample and place it on ice. Process additional samples, or clean the sonicator probe per your lab's standard practices, and return the Tube Cooler to the  $-20^{\circ}$ C freezer.

# **Appendix**

# Section A. Attaching the Alternate Spring Bracket

The alternate spring bracket was provided in case you need to remove the converter/probe bracket from the top of the rod, which you would need to do if your converter/probe is too large to be held by the bracket. The alternate bracket enables you to reattach the continuous force spring so that it can support the weight of the platform when it is moved up and down.

To replace the converter/probe bracket with the alternate spring bracket, the rod and platform will need to be removed from the sound enclosure. To do this, lay the sound enclosure box flat with the door facing up and open.

Loosen the screw on the top of the box that holds the rod in place using the supplied hex wrenches (top image). Then remove the three screws and bracket supporting the rod on the bottom of the sound enclosure (bottom image). Gently slide the rod down 1-2 inches through the hole in the bottom of the sound enclosure to bring the top of the rod below the interior insulation. Then angle the top of the rod into the probe chimney and gently slide the rod up until the entire platform is released from the sound enclosure. With the platform removed from the sound enclosure, follow the instructions to replace the converter/probe bracket.





Loosen the two set screws on the front of the bracket, then loosen the small set screw on top of the bracket. You can then slide out the bolt and bobbin around which the continuous force spring is wound (top image). Be sure to hold the spring assembly firmly to prevent it from unwinding from the bobbin, which could damage it. Take care with the metal edges of the spring, as they could potentially cut your finger.

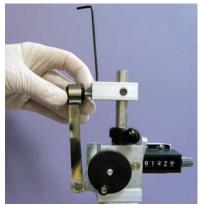
While you are holding the spring/bobbin/bolt, slide the converter/probe bracket off the top of the rod and replace it with the alternate spring bracket. You can put the spring assembly into the new bracket (middle image).

Use one of the two large set screws that were in the converter/probe bracket to secure the alternate spring bracket to the rod. Likewise, use the small set screw that was in the converter/probe bracket to secure the spring in the alternate spring bracket.

You can then adjust the height of the bracket to where you would like it to be and firmly secure the set screw. If you will need to secure some other kind of bracket to hold an oversized converter/probe, you will need to place the bracket as low as it will go on the rod (lower image).

To reassemble the platform back inside the sound enclosure, angle the top of the rod into the probe chimney. Gently slide the bottom of the rod through the hole in the bottom of the sound enclosure. Align the top of the rod into the designated hole at the top of the sound enclosure. Make sure the platform faces the interior of the box with enough clearance for the door to close. Secure the rod in place by tightening the screw on the top of the sound enclosure and reassembling the three screws and bracket on the bottom of the sound enclosure. Set the EpiShear Cooled Sonication Platform upright and proceed to Calibration of the Platform Counter on page 6.







### Section B. Related Products

Sonication	Format	Catalog No.
EpiShear™ Probe Sonicator	110V	53051
•	230V	53052
EpiShear™ 5/64" (2 mm) Sonicator Probe	1 probe	53056
EpiShear™ 1/8" (3.2 mm) Sonicator Probe	1 probe	53053
EpiShear™ 1/4" (6.4 mm) Sonicator Probe	1 probe	53057
EpiShear™ Cooled Sonication Platform, 1.5 ml	1 platform	53080
EpiShear™ Cooled Sonication Platform, 15 ml	1 platform	53081
EpiShear™ Cooled Sonication Platform, 50 ml	1 platform	53082
Support Stand / Converter Clamp	1 unit	53054
Sound Enclosure	1 enclosure	53060
Q800R Sonicator	110V	53062
	230V	53063

ChIP-IT™ Kits	Format	Catalog No.
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009
ChIP-IT™ Express Shearing Kit	10 rxns	53032
ChIP-IT™ Express Enzymatic Shearing Kit	10 rxns	53035
ChIP-IT™ Express HT	96 rxns	53018
Re-ChIP-IT™	25 rxns	53016
RNA ChIP-IT™	25 rxns	53024
Chromatin IP DNA Purification Kit	50 rxns	58002
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014
Siliconized Tubes, 1.7 ml	25 tubes	53036
ChIP-IT™ Control Kit – Human	5 rxns	53010
ChIP-IT™ Control Kit – Mouse	5 rxns	53011
ChIP-IT™ Control Kit — Rat	5 rxns	53012
RNA ChIP-IT™ Control Kit – Human	25 rxns	53024
Ready-to-ChIP HeLa Chromatin	10 rxns	53015
Ready-to-ChIP Hep G2 Chromatin	10 rxns	53019
Ready-to-ChIP K-562 Chromatin	10 rxns	53020
Ready-to-ChIP NIH/3T3 Chromatin	10 rxns	53021
Bridging Antibody for Mouse IgG	500 µg	53017

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#### ChIP Control qPCR Primer Sets

For an up-to-date list of over 30 qPCR Primer Sets, please visit www.activemotif.com/chipprimers.

Modified Histones Array	Format	Catalog No.
MODified™ Histone Peptide Array	1 array	13001

#### **Histone Antibodies**

For an up-to-date list of over 150 antibodies against histones and modified histones, please visit www.activemotif.com/histoneabs.



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