

DNA Shearing Quick Guide:

M220 Focused-ultrasonicator and Holder XTU

This Quick Guide provides DNA Shearing protocols with the Covaris M220 Focused-ultrasonicator. For optimal DNA Shearing performances at each specified volume, universal Holder XTU should be used with the correct combination of microTUBE and Insert. Holder miniTUBE should be used with miniTUBE.

15 µl sample volume- from 150 to 550 bp

Insert: M220 Holder XTU Insert microTUBE 15 µl (PN 500420)

Vial: microTUBE-15 AFA Beads Screw-Cap (PN 520145)

| Target BP (Peak) | 150 | 250 | 350 | 550 |
|-------------------------|-----|-----|-----|-----|
| Peak Incident Power (W) | 30 | 30 | 30 | 30 |
| Duty Factor | 20% | 20% | 20% | 20% |
| Cycles per Burst | 50 | 50 | 50 | 50 |
| Treatment Time (s) | 250 | 80 | 42 | 23 |
| Temperature (°C) | 20 | 20 | 20 | 20 |
| Sample volume (µl) | 15 | 15 | 15 | 15 |



To ensure reproducible DNA shearing, it is required to centrifuge samples before processing DNA in a microTUBE-15. Please read Appendix A for detailed instructions.

50 µl sample volume- from 150 to 800 bp

Insert: M220 Holder XTU Insert microTUBE 50 µl (PN 500488)

Vial: microTUBE-50 AFA Fiber Screw-Cap (PN 520166)

| Target BP (Peak) | 150 | 200 | 250 | 300 | 350 | 400 | 550 | 800 |
|-------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Peak Incident Power (W) | 75 | 75 | 75 | 75 | 75 | 75 | 75 | 75 |
| Duty Factor | 10% | 10% | 10% | 10% | 10% | 10% | 10% | 5% |
| Cycles per Burst | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| Treatment Time (s) | 360 | 195 | 130 | 90 | 70 | 60 | 40 | 50 |
| Temperature (°C) | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| Sample volume (µl) | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |



Even if the Water Level check button is green in SonoLab, please check that water is in contact with Insert when using microTUBE-50.

130 µl sample volume- from 150 to 1,500 bp

Insert: M220 Holder XTU Insert microTUBE 130 µl (PN 500489)

Vial: microTUBE AFA Fiber Snap-Cap (PN 520045)

| Target BP (Peak) | 150 | 200 | 300 | 400 | 500 | 800 | 1,000 | 1,500 |
|-------------------------|-----|-----|-----|-----|-----|-----|-------|-------|
| Peak Incident Power (W) | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Duty Factor | 20% | 20% | 20% | 10% | 10% | 5% | 2% | 2% |
| Cycles per Burst | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| Treatment Time (s) | 330 | 150 | 65 | 70 | 50 | 52 | 90 | 20 |
| Temperature (°C) | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| Sample volume (µl) | 130 | 130 | 130 | 130 | 130 | 130 | 130 | 130 |




200 µl sample volume- 2,000; 3,000 and 5,000 bp

Holder: M220 Holder miniTUBE (PN 500302)

Vials: miniTUBE Clear (PN 520064) or miniTUBE Blue (PN 520065) or miniTUBE Red (PN 520066)

| Target BP (Peak) | 2,000 | 3,000 | 5,000 |
|-------------------------|-------|-------|-------|
| miniTUBE | Clear | Blue | Red |
| Peak Incident Power (W) | 8 | 6 | 8 |
| Duty Factor | 20% | 20% | 20% |
| Cycles per Burst | 1,000 | 1,000 | 1,000 |
| Treatment Time (s) | 900 | 600 | 600 |
| Temperature (°C) | 7 | 20 | 20 |
| Sample Volume (µl) | 200 | 200 | 200 |



To fragment DNA to size larger than 5 kb, Covaris offers the **g-TUBE**: a single-use device that shears genomic DNA into selected fragments sizes ranging from 6 kb to 20 kb. The only equipment needed is a compatible bench-top centrifuge.

Values mentioned in this Quick Guide are nominal values. The tolerances are as follow:

- Temperature +/-2°C
- Sample volume
 - o microTUBE-15: from 15 to 20 µl, +/- 1 µl
 - o microTUBE-50: 50 µl, +/- 3 µl
 - o microTUBE Snap-Cap: 130 µl, +/- 5 µl
 - o miniTUBE: 200 µl, +/- 10 µl

Sample preparation guidelines:

- **DNA input:** From 100 ng to 5 µg purified DNA (1 µg for the microTUBE-15)
- **Buffer:** Tris EDTA, pH 8.0
- **DNA quality:** Genomic DNA (> 10 kb). For lower quality DNA, Covaris recommends setting up a time dose response experiment for determining appropriate treatment times.

Recommended settings are subject to change without notice.

See following link <http://www.covarisinc.com/resources> for updates to this document.

Supplies

| | | |
|----------------------------|--|--------|
| Covaris AFA Tubes | microTUBE-15 AFA Beads Screw-Cap (25) | 520145 |
| | microTUBE-50 AFA Fiber Screw-Cap (25) | 520166 |
| | microTUBE AFA Fiber Snap-Cap (25) | 520045 |
| | miniTUBE Clear (25) | 520064 |
| | miniTUBE Blue (25) | 520065 |
| | miniTUBE Red (25) | 520066 |
| Preparation station | microTUBE Prep Station Snap & Screw Cap | 500330 |
| Holder | M220 Holder XTU | 500414 |
| | M220 Holder miniTUBE | 500302 |
| Inserts | M220 Holder XTU Insert microTUBE 15 μ l | 500420 |
| | M220 Holder XTU Insert microTUBE 50 μ l | 500488 |
| | M220 Holder XTU Insert microTUBE 130 μ l | 500489 |
| Starter Kit | M220 NGS 15 to 130 ul microTUBE Starter Kit Includes: M220 Holder XTU M220 Holder XTU Insert microTUBE 15 μ l M220 Holder XTU Insert microTUBE 50 μ l M220 Holder XTU Insert microTUBE 130 μ l microTUBE-15 AFA Beads Screw-Cap (25) microTUBE-50 AFA Fiber Screw-Cap (25) microTUBE AFA Fiber Snap-Cap (25) Centrifuge microTUBE Adapters (25) microTUBE Prep Station Snap & Screw Cap | 500417 |
| | M220 NGS miniTUBE Starter Kit Includes: M220 Holder miniTUBE miniTUBE Clear (25) miniTUBE blue (25) miniTUBE red (25) miniTUBE Prep Station & Stand | 500297 |
| Instrument cleaning | M220 Fill & Drain Accessory Kit | 500299 |
| | M220 Swab Cleaning Kit | 500298 |
| AFA-grade Water | 800 ml of AFA-grade water | 520101 |
| Centrifuge Adapter | Centrifuge microTUBE Adapters (25) | 520059 |

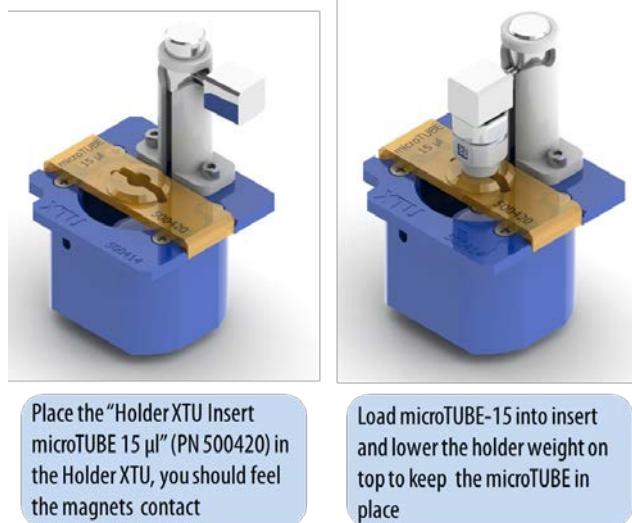
Appendix A – Using microTUBE-15 with Holder XTU

1. Sample loading and centrifugation



2. Sample processing

Load microTUBE-15 as described and use settings provided in page 1



3. Sample recovery

