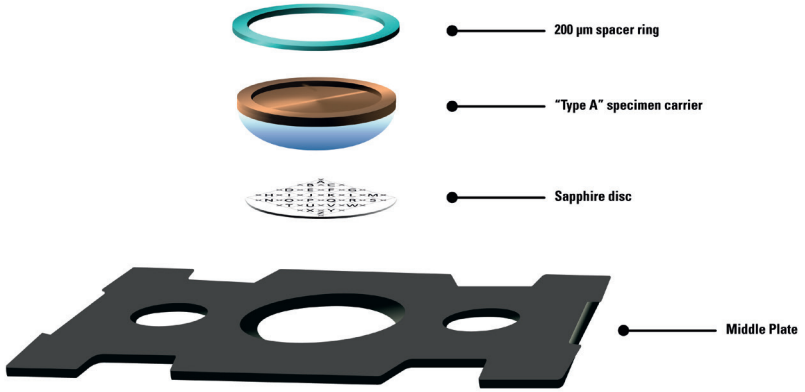
Leica
MICROSYSTEMS

DESCRIPTION FOR SAPPHIRE FREEZING

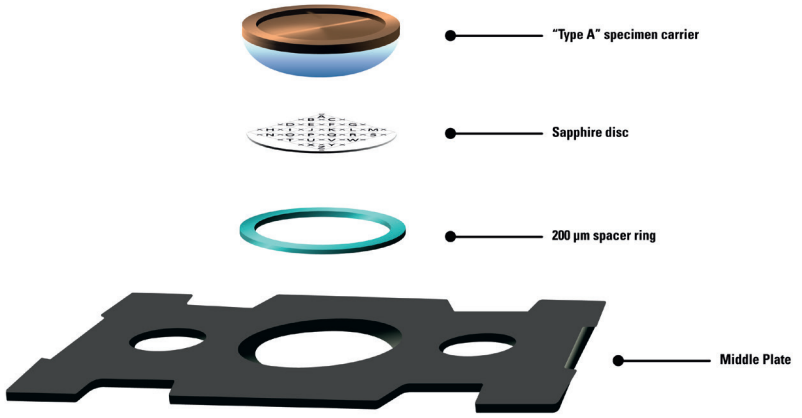
Method 1
Sapphire – 6 mm flat carrier A with 100 µm side down – 200 µm spacer ring

1. Arrange all the sapphires and rings you need and check if the 100 µm spacer ring that you will use between the sapphires is completely flat.
2. Take out sapphire in horizontal position.
3. Hold down a filter paper and quickly blot dry the bottom surface of the sapphire. Do this fast to not dry out the cells on top.
4. Hold the sapphire 45 degrees and align the edge of the sapphire within the recess of the middle plate and it should fall in nicely.
5. Normally you will always transfer enough medium on the sapphires You can always add more medium with a pipette. Only, do not scratch the surface of the sapphire.
6. Take a flat carrier "type A" with the 100 µm cavity facing downwards and only wet this side in e.g. hexadecane or medium. Shift it sideways to assure that there won't be any air bubbles.
7. Add the 200 µm spacer ring.
8. Blot away the excess. Important here is that you blot fast and put some pressure to prevent shifting of the 200 µm spacer ring. The space between the carrier and middle plate recess should be wet. One quick blot should be enough.
9. Shift the middle plate on the half cylinder and freeze.



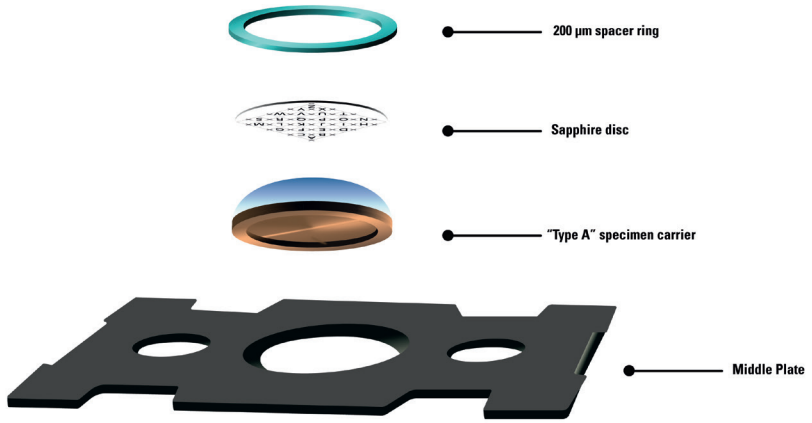
Method 2
Sapphire – 200 µm spacer ring - 6mm flat carrier A with 100 µm side down

1. Place the 200 µm spacer ring on the bottom of the middle plate.
2. Add the sapphire disc as described above.
3. Take a flat carrier "type A" with 100 µm cavity facing downwards and only wet this side in e.g. hexadecane or medium. Shift it sideways to assure that there won't be any air bubbles.
4. Blot the excess away as described above.
5. Shift the middle plate on the half cylinder and freeze.



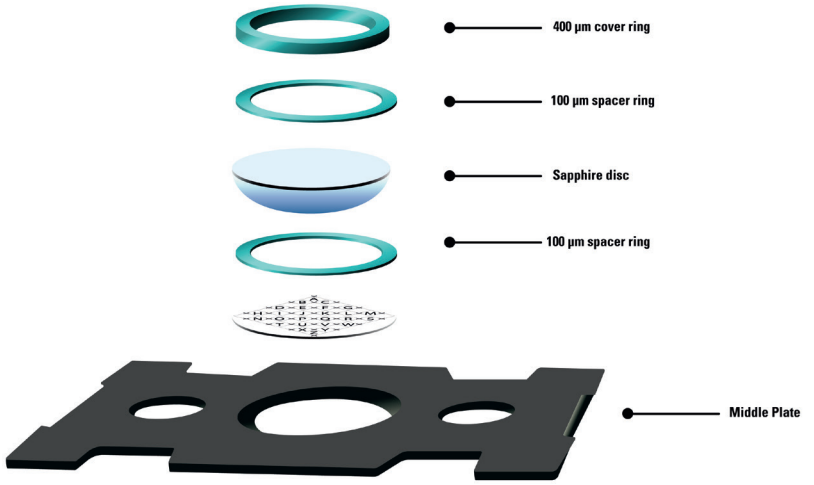
Method 3
6 mm flat carrier A with 100 µm side up - Sapphire – 200 µm spacer ring

1. Place a flat carrier "type A" with 100 µm cavity up inside the middle plate. Add media to the carrier.
2. Take out sapphire in horizontal position and invert them so that the cells are facing down. Do this as fast as possible.
3. Hold the sapphire 45 degrees and align the edge of the sapphire within the recess of the carrier and let it fall down slowly.
4. Add the 200 µm spacer ring.
5. Blot the excess away as described above.
6. Shift the middle plate on the half cylinder and freeze.



Method 4
Sapphire – 100 µm spacer ring – Sapphire – 100 µm spacer ring – 400 µm cover ring

1. Arrange all the sapphires and rings you need and check if the 100 µm spacer ring that you will use between the sapphires is completely flat.
2. Add the sapphire disc as described above
3. Add a 100 µm spacer ring and press it down with tweezers.
4. Take the other sapphire and slide it above it, then press it down.
5. Add the 100 µm spacer ring and 400 µm cover ring.
6. Once added, hold down the sapphire sandwich with tweezers and preferably use a thick filter paper to blot away the liquid on the sapphire. Do not blot away everything, as it will dry out the sapphire sandwich. There should be still liquid in between the rings.
7. Shift the middle plate on the half cylinder and freeze.



Note:
- > **Assure that you do not mix the 200 µm and 100 µm spacer rings**
- > **The tweezers with blue isolation are not magnetized when delivered but when touching the magnet in the cryo box they become magnetized.**