**SOP of synthesis and handling of heavy metal contained nanoparticles:**

**General notice:**

1. Use dedicated “heavy metal fume hood”
2. Never pouring any heavy metal contained solution or other hazard solution, always use syringes or pipettes to transfer solution.
3. Don’t put any wet heavy metal contained stuff direct into the trash bin, keep them in the fume hood until dry before trashing
4. Lab coats, safety glasses, and gloves are a must during the experiment. Lab coats are to be placed in the cuboard dedicated for heavy metal lab coats
5. Heavy metal solution or CMR substances outside the lab must be carried in double containment. Cuvettes must be sealed and be placed in cuvette holders, inside a second container, e.g. plastic tray. Cyvettes, vials or beakers that do not air tight sealed are not permitted with liquid CMR substances.
6. These instructions assume that the product is either a suspension or thin film. Nano particles that are produced in these processes must be strongly bound into large clusters or any surface. It must be thus insured that these particles are never loose in powder form.

**Prepare before beginning:**

1. Preparing at least two trash bins in the fume hood for waste handling. Line one with glove and paper to reduce exposure during cleaning and use for pipette tips and syringed. Use second trash to hold the capped needle tips.
2. Prepare a beaker containing spare needles and syringe tips
3. For high temperature (> 100 oC) experiment, metal heating mantel or other director heater must be used that is to remain in heavy metal fume hood
4. Prepare heating equipment on lab jack under the reaction vessel
5. Place lab jack and all liquid containers in plastic tray inside fume hood (containment)
6. Prepare and label one single use glas vial per substance
7. If DMF is used, prepare proper second pair of gloves
8. Prepare tweezer for handling of magnets and small container to keep them. Prepare small amount of DMSO for washing of magnets
9. Prepare holder for centrifuge tube or vials that will receive the sample
10. Inspect sample receiving vessels and all glassware for potential damages.
11. Prepare solvent trash (for washing)

**For work under oxygen free environment additionally prepare:**

1. Use three (or more) neck glass vessel dedicated for heavy metal synthesis.
2. Seal all but middle neck with suitable septums (careful, standard rubber septa have 85degree upper temperature limit)
3. Place temperature sensor in suitable septum
4. Place attach middle neck to condenser and start the cooling water before work is commenced
5. Connect nitrogen over acid bubbler one of the three necks
6. Connect second bubbler as output on top of condenser.
7. Place heating equipment on lab jack under round bottle flask
8. Prepare vessel for cooling equipment (if required) and separate “clean” container that is not stored in fume hood for getting the ice

**Detailed experiment procedures and related SOP:**

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|  | Experiment procedure | SOP |
| 1 | Weighing the heavy metal power, put it in the container | * Wair breathing mask and nitril gloves
* All the spoons, weighting papers and containers are dedicated and labeled for heavy metal or CMR usage
* Place powder in dedicated (labeled) single use glas container
* After weighing, wipe scale and area around twice, first with water to bind particles, second with small amount of DMSO. Use tweezers to hold the paper during wiping, move heavy metal trash bin under weighing table and trash paper directly.
* If magnetic stirring is needed. Stirring bars must be put into the vial before the power and solvent.
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| 2 | Dissolve the powers in the fume hood for anion precursors. Using syringe and needels to take the solvent and inject into the vial.  | * Avoid to use the toxic solvent such as DMF with DMSO as alternatives. If DMF has to be used prepare replacement form.
* If magnetic stirring is needed. Stirring bars must be put into the vial before the power and solvent. Use dedicated magnets only for heavy metals, if a new magnet is used it has to stay in the fume hood.
* Take on second pair of gloves
* Transfer solvent with fresh pipette into single use glas container with solvent and mix carefully using the pipette
* Eject the pipette tips into the prepare trash
* Inject the solution into the reaction vessel using fresh syringe and needle.
* Cap the needle immediately.
* Remove the needle with the cap (do not touch the needle directly!)
* Place needle and syringe dedicated trash
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| 3 | Preparing cat-ion precursors in three neck flask in N2 atmosphere with heating | * No large volume reaction (> 100 ml) is allowed, a suggested typical volume should be less than 20 ml.
* Inject precursor, being careful not to touch the potentially contaminated septum
* Special stopper for High Temperature (above 85 deg) must be used.
* Flow the nitrogen for a little time to insure oxygen free environment.
* engage heater and check that temperature sensor is showing the right temperature.
* Re-check that cooling water is flowing in the condenser
* Recheck that output bubbler is working properly and that an oxygen free environment is reached
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| 4 | Injection of anion precursor in to cation precursor at HT | * Inject anion precursor using fresh needle. Take care to not touch the contaminated septum or needle
* Directly cap the needle and remove the needle touching the cap only.
* Place capped needle and syringe in prepare trash
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| 6 | Cooling and ceasing the reaction  | * Stop the heater
* Using lab jack to lower the position of the heater
* Remove the heater and put the ice bath or water bath on the jack (ice need to be taken in other container from the fridge)
* Lifting the jack so the ice bath contacts the flask for cooling
* Once cold, remove the cooling and place the aluminium heat mantel on the lab jack again.
* Remove the clamper between the condenser and the flask
* Lower the lab jack so that the flask is removed from the condenser and top outlet is opened.
* Move the lab jack so that at no time solvent that could drop from the condenser could hit the hand. Make sure that possible drops hit inside the plastic containment. Potentially use tweezer and paper to dry opening and place paper in pipette trash.
* Using pipette or needles to take out the samples and transfer to the vials or centrifuge tubes without holding the tubes!
* Wait a few seconds to let small droplets dry
* Seal centrifuge tube/vial.
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| 6 | Centrifuge the obtained solutions | Only use sealed centrifuge tubes to take sample outside of fume hood. Be sure the weight in the centrifuge is balanced before start the centrifuge.  |
| 7 | Remove sample from centrifuge tubes | Use holder to hold centrifuge tubes and vials/cyvettestransfer with pipettes/syringetrash pipettes/needles directly after use |
| 8 | Cleaning | Wait for a few minutes to let droplets dry, stop N2 flow, stop cooling water flow.take fresh double pair of gloves.After last action wait for a minute to let small droplets dry!Once dry remove septums carefully to not get exposed. Wash septums carefully in beaker using DMSO and tweezers and transfer liquids via pipette into solvent trash. wash the reaction vessel 2x!!!using DMSO, transferring it via pipette into solvent trash trash the glove with the pipette tips and syringes, the single use glas ware and the needles into the appropriate containersIf additional washing is required make sure that only pieces that have been multiple times flushed shall be taken in gloved hand. At no time must anything that has touched CMR substances directly must be in contact with the glove.  |