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:

	Experiment procedure	SOP	
1	Weighing the heavy	\checkmark	Wair breathing mask and nitril gloves
	metal power, put it in the	\triangleright	All the spoons, weighting papers and containers are dedicated
	container		and labeled for heavy metal or CMR usage
		\triangleright	Place powder in dedicated (labeled) single use glas container
		\triangleright	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.
2	Disselve the new one in	~	Avaid to use the toxic columnt such as DNAE with DNASO as
2	the fume head for anion		Avoid to use the toxic solvent such as DIVIF with DIVISO as
		Δ	If magnetic stirring is peeded. Stirring bars must be put into the
	Using syringe and	Í	vial before the power and solvent. Use dedicated magnets only
	needels to take the		for heavy metals, if a new magnet is used it has to stay in the
	solvent and inject into		fume hood.
	the vial.	\succ	Take on second pair of gloves
		\triangleright	Transfer solvent with fresh pipette into single use glas container
			with solvent and mix carefully using the pipette
		\succ	Eject the pipette tips into the prepare trash
		\triangleright	Inject the solution into the reaction vessel using fresh syringe
			and needle.
		\succ	Cap the needle immediately.
			Remove the needle with the cap (do not touch the needle
		~	directly!)
			Place needle and syringe dedicated trash
3	Preparing cat-ion	≻	No large volume reaction (> 100 ml) is allowed, a suggested
	precursors in three neck		typical volume should be less than 20 ml.
	flask in N2 atmosphere	\triangleright	Inject precursor, being careful not to touch the potentially
	with heating		contaminated septum
		۶	Special stopper for High Temperature (above 85 deg) must be
			used.
			Flow the nitrogen for a little time to insure oxygen free
		2	environment.
		ĺ.	the right temperature.
		≻	Re-check that cooling water is flowing in the condenser
		\triangleright	Recheck that output bubbler is working properly and that an
			oxygen free environment is reached
4	Injection of anion	\triangleright	Inject anion precursor using fresh needle. Take care to not
	precursor in to cation		touch the contaminated septum or needle
	precursor at HT		Directly cap the needle and remove the needle touching the
			cap only.
	- H	>	Place capped needle and syringe in prepare trash
6	Cooling and ceasing the	>	Stop the heater
	reaction	\succ	Using lab jack to lower the position of the heater

		Remove the heater and put the ice bath or water bath on the	
		iack (ice need to be taken in other container from the fridge)	
		Lifting the jack so the ice bath contacts the flask for cooling	
		Children and the sealing 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.	
6	Centrifuge the obtained	Only use sealed centrifuge tubes to take sample outside of fume	
	solutions	hood. Be sure the weight in the centrifuge is balanced before start	
		the centrifuge.	
7	Remove sample from	Use holder to hold centrifuge tubes and vials/cyvettes	
	centrifuge tubes	transfer with pipettes/syringe	
	_	trash 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 ninette tins and syringes, the single use	
		glas ware and the needles into the appropriate containers	
		If 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.	