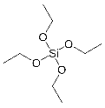
Title	#2.1
	BOMB coating ferrite MNPs with silica oxide
Keywords	magnetic nanoparticles, SiO_2, magnetic separation, silica coating
Authors	Oberacker P*, Stepper P*, Bond DM*, Höhn S, Focken J, Meyer V, Schelle L, Sugrue VJ, Jeunen GJ, Moser T, Hore SR, von Meyenn F, Hipp K, Hore TA# and Jurkowski TP#
Citation	*Oberacker et al., Bio-On-Magnetic-Beads (BOMB): Open platform for high-throughput nucleic acid manipulation. PLOS Biology,17(1), https://doi.org/10.1371/journal.pbio.3000107*
Online	https://bomb.bio/protocols/
Revision	V1.4 (20th April 2020)

Summary

Here we provide a simple protocol for silica-coating of ferrite MNPs (BOMB protocol #1.1). The silica-coated magnetic beads are synthesised by a modified protocol including the hydrolysis of tetraethyl orthosilicate (TEOS) on the surface of ferrite magnetic core particles according to Stöber et al. 1968 [1].

A more detailed version of this protocol was published with bio-protocol [2].



TEOS - $Si(OC_2H_5)_4 + 2\ H_2O \rightarrow SiO_2 + 4\ C_2H_5OH$

Chemicals

Name	Provider	PN	MW [g/mol]	Safety codes	
Ethanol (C_2H_6O, 99.9 %)	Riedel-de Haën	34963	46.07	 Danger	H: 225-319 P: 210-280-305+351+338-308+313
Tetraethyl orthosilicate (≥99%) (GC)	Aldrich	86578	208.33	 Danger	H: 226+319+332+335
Ammonia solution (NH_4OH, 25%)	EMD Millipore	1.05432	n.a.	 Corrosive Danger	H: 290+314+335+400 P:273+280+301+330+331+305+351+338+308+310

Please consult appropriate MSDS information before working with these chemicals! Use lab coat, gloves and eye protection at all times! The chemicals are available from other providers as well. No preference is given to the indicated vendors.

Equipment and setup

Fume hood

Heated magnetic stirrer (e.g. IKAMAG REO)

Strong neodymium permanent magnet (e.g. NdFeB N45 40x40x20 mm)

Sterile plastic bottles

BOMB Silica-coating

Step	Task	Time	☑
⚠	*All procedures can be performed under inert N_2 atmosphere or atmospheric oxygen conditions. The protocol will work in both cases, however, the formation of brown precipitate (iron oxide) can be reduced or eliminated when working under N_2 atmosphere and therefore, the stability of the uncoated beads can be increased.*		
1	Prewash 22.5 g (wet mass ~1.2 g dry) of magnetic core particles with ethanol	5 min	☐
2	Mix 2 L of 99% ethanol with 50 ml of 25% ammonia solution and ~22.5 g (wet mass) of the synthesized iron oxide MNPs (A) in a heat resistant 2.5 L bottle using a magnetic stirrer (300-400 rpm). Switch on the heating and allow the solution to heat up to ~80 °C.	30 min	☐
⚠	*Note: It is very important that the core particles are efficiently distributed in the solution. Otherwise, large clusters of MNPs are coated with silica, leading to a decreased performance of the beads in nucleic acid capture applications.*		
3	Add 45 ml Tetraethyl orthosilicate (TEOS) under constant stirring and incubate for another 30 minutes	30 min	☐
⚠	*Note: The size of the particles can be controlled using different ratios of core particles and TEOS. The standard ratio used in the above protocol is: 1 g of paramagnetic core particles to 2 ml TEOS, which results in particles with an average size of ~400 nm. Generally, more TEOS yields larger beads*		
4	Add 400 ml of ddH_2O to the solution		☐
5	Allow the reaction to proceed for >4 h (ideally overnight)	4 hours to o/n	☐
⚠	*Note: TEOS hydrolyzes spontaneously in water which will result in white silica precipitates which do not contain a paramagnetic core. If large amounts of this precipitate forms, use fresh TEOS for the reaction.*		
7	Cool down the solution to RT	15 min	☐
8	Separate the coated MNPs using a strong neodymium magnet.	5 min	☐
	Note: Uncoated MNPs get slowly oxidized over time which is indicated by a brown color of the supernatant after magnetic separation.		
9	Wash twice with pure water	15 min	☐☐
10	Wash twice with pure ethanol	15 min	☐☐
11	Wash with pure water until the pH of the solution becomes neutral (3-4 times)	30 min	☐☐
⚠	*Note: To measure the synthesis yield, magnetically pellet the silica beads, remove the water, and weigh the product wet mass. The coated beads can be stored at RT for at least 1 year.*		
End	Check the yield by weighing the wet mass of the beads	**~12 h** (2 h hands-on)	
	Store @ RT for up to 1 year		

Modifications

By using different ratios of core particles and TEOS one can control the thickness of the glass layer and consequently the size of the particles. The standard ratio used in the above protocol is: 1 g of magnetic core particles (wet) to 2 ml TEOS, which results in particles with an average size of ~400 nm. Increasing or decreasing the TEOS to beads ratio results in formation of other sizes of coated particles.

Troubleshooting

Problem	Solution
Brown colour of the reaction, low yield of beads retained	• The uncoated magnetic core particles get slowly oxidized during storage, prepare fresh core particles and redo the reaction
Large amounts of white precipitate	• TEOS hydrolyses spontaneously in water solution, thus forming silica nanoparticles. Use fresh TEOS for the reaction

Exemplary results

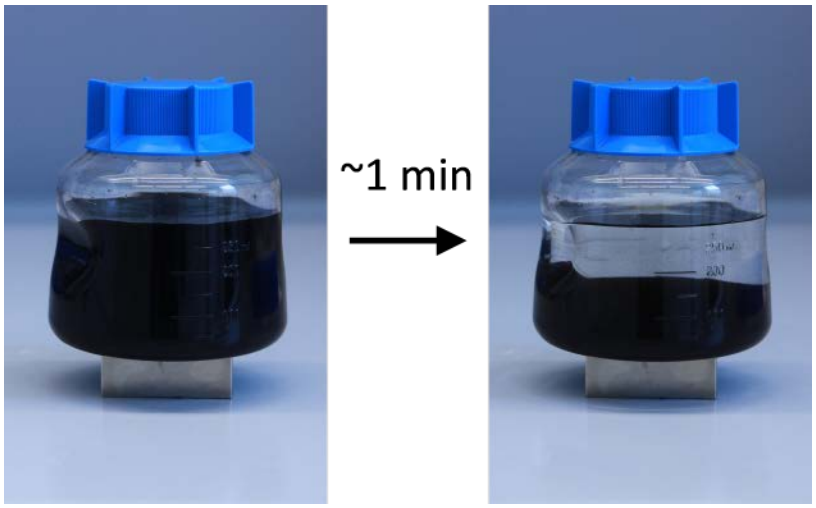


Fig 1: Silica coated MNPs. *Magnetic decantation happens within a minute. The water appears clear indicating no iron oxidation occurring during storage.*

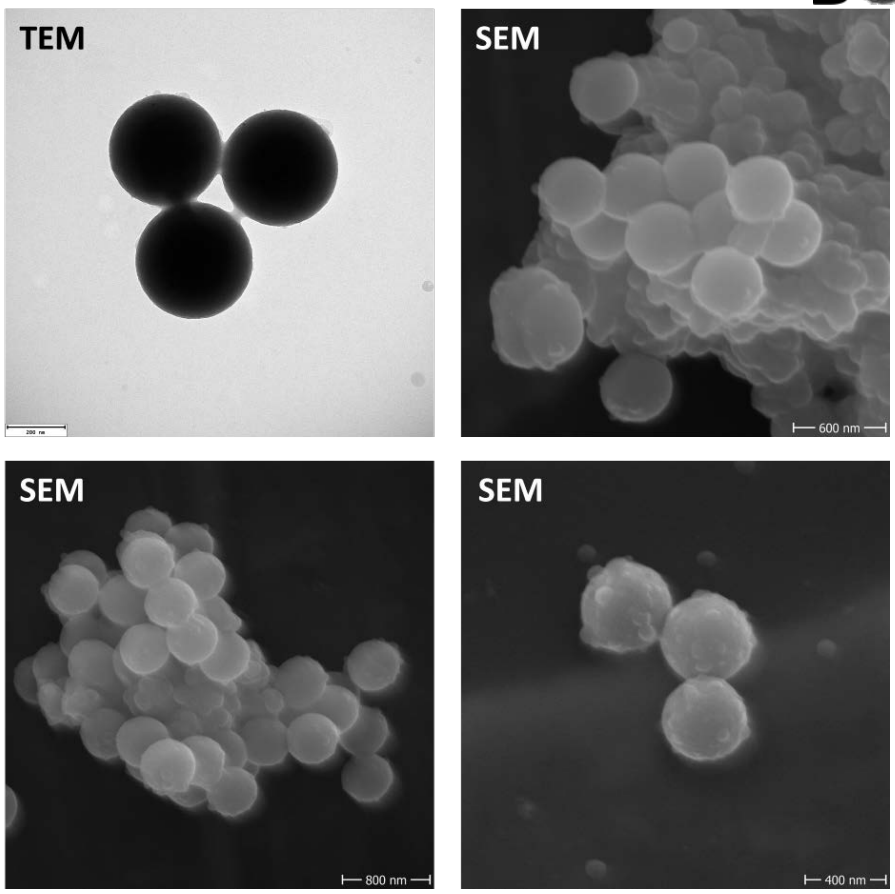


Fig 2:. Electron micrographs of silica coated MNPs.

References

1. Stöber W, Fink A, Bohn E. Controlled growth of monodisperse silica spheres in the micron size range. J Colloid Interface Sci. 1968;26: 62–69. doi:10.1016/0021-9797(68)90272-5

2. Oberacker P, Stepper P, Bond D, Hipp K, Hore T, Jurkowski T. Simple Synthesis of Functionalized Paramagnetic Beads for Nucleic Acid Purification and Manipulation. Bio-Protocol. 2019;9: 1–10. doi:10.21769/bioprotoc.3394