




<b>Protocol</b>	#3.1
<b>Title</b>	<b>BOMB coating ferrite MNPs with carboxyl groups (methacrylic acid)</b>
<b>Keywords</b>	magnetic nanoparticles, magnetic separation, carboxyl-coating, methacrylic acid
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<b>Citation</b>	Oberacker et al.(2019), Bio-On-Magnetic-Beads (BOMB): Open platform for high-throughput nucleic acid manipulation. PLOS Biology,17(1), <a href="https://doi.org/10.1371/journal.pbio.3000107">https://doi.org/10.1371/journal.pbio.3000107</a>
<b>Online</b>	<a href="https://bomb.bio/protocols/">https://bomb.bio/protocols/</a>
<b>Revision</b>	V1.0 (15 <sup>th</sup> August 2018)

## Summary

Here we describe a simple protocol for coating ferrite magnetic nanoparticles (MNPs, BOMB protocol #1.1) with carboxyl groups. The carboxyl-coated magnetic nanoparticles are synthesized by the classical radical polymerisation of methacrylic acid (MAA) on the surface of ferrite magnetic core particles (protocol modified from Yu et al. [1]).

## Chemicals

Name	Provider	PN	MW [g/mol]		Safety codes
<b>Ethanol (C<sub>2</sub>H<sub>6</sub>O, 99.9 %)</b>	Honeywell/ Riedel-de Haën	34963	46.07	 Danger	H: 225-319 P: 210-280- 305+351+338-308+313
<b>Methacrylic acid (≥99%) (GC)</b>	Aldrich	155721	86.09	 Danger	H: 302+332-311-314-335 P:260-280- 301+312+330- 303+361+353-304- 340+310-305+351+338
<b>Potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, ≥99%)</b>	Sigma-Aldrich	216224	270.32	 Danger	H: 272-302-315-317- 319-334-335 P: 220-261-280- 305+351+338-342+311

*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

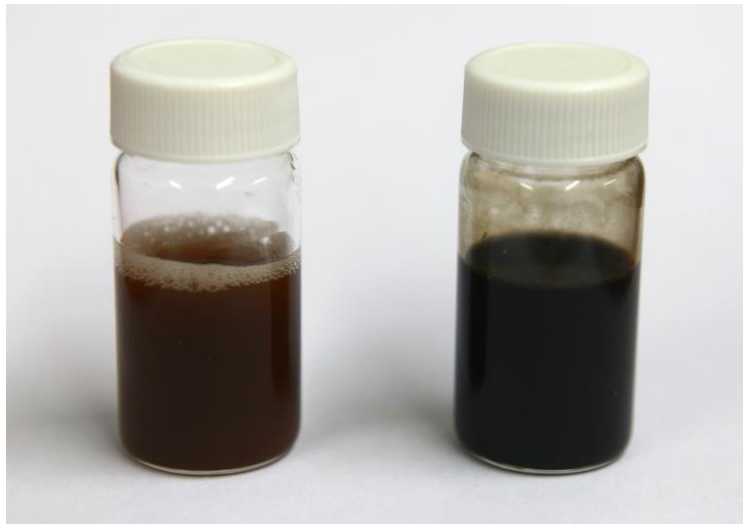
## BOMB carboxyl-coating

Step	Task	Time	<input checked="" type="checkbox"/>
	<i>All procedures can be performed under inert N<sub>2</sub> atmosphere or atmospheric oxygen conditions</i>		
1	Add 1 g (wet mass) of the synthesized iron oxide MNPs dispersed in 45 ml of water and 1.15 g sodium dodecyl sulfate to 200 ml of purified, degassed water in a 250 ml flask with stirring	5 min	<input type="checkbox"/>
2	Heat up the reaction solution to 70 °C	30 min	<input type="checkbox"/>
3	Add 0.96 ml of MAA into the flask. The pH value decreases to about 3	5 min	<input type="checkbox"/>
4	Equilibrate the reaction mixture for about 45 min while keeping the temperature	45 min	<input type="checkbox"/>
5	Add 1.98 g of initiator K <sub>2</sub> S <sub>2</sub> O <sub>8</sub> to the solution	15 min	
6	Let the polymerization reaction progress at 70 °C for 2 h	2 hours	<input type="checkbox"/>
7	Separate the coated MNPs using a strong neodymium magnet	15 min	<input type="checkbox"/> <input type="checkbox"/>
8	Afterwards cool the reaction to room temperature	15 min	<input type="checkbox"/> <input type="checkbox"/>
9	Remove free MAA and PMAA from the coated magnetic particles by a magnetic particle concentrator at room temperature	30 min	<input type="checkbox"/>
10	Disperse the isolated magnetic nanoparticles in deionized water in an ultrasonic bath, followed by magnetic extraction	5 min	<input type="checkbox"/>
11	Wash the beads with ddH <sub>2</sub> O at least 5 times or until the detergent is completely removed	1 h	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>
12	Dispersed the magnetic particles in 250 ml of deionized water	5 min	
<b>End</b>	Check the yield by weighing the wet mass of the beads	~7 h (2 h hands-on)	
	Store @ RT for up to 1 year		

## Troubleshooting

Problem	Solution
Particles are not magnetic	Make sure that the solution is degassed. Use freshly prepared magnetic core particles

## Exemplary Results



*Fig 1: Carboxyl-coated MNPs appear brownish (left vial) when compared to silica-coated MNPs (right vial, BOMB protocol #2.1)*

## References

1. Yu S, Chow CM. Carboxyl group (-CO<sub>2</sub>H) functionalized ferrimagnetic iron oxide nanoparticles for potential bio-applications. *J Mater Chem*. Royal Society of Chemistry; 2004;14: 2781–2786. doi:10.1039/b404964k