Samples and Measurement Protocol

Sample tubes

All samples should be inserted in a PCR tube of 200 µL.

The following PCR tube is compatible with our automated Pepric Particle Spectrometer, PPS4S-AS.

Axygen® PCR microtubes with domed cap 0.2 mL (Product number: PCR-02D-C)

No sample preparation is required

It is not necessary for a pEPR measurement to perform any sample preparation. On the contrary, sample preparation is not only time consuming, it is also an extra source of sampling and manipulation errors adding up to the total (in)-accuracy of the measurement. So better not to homogenize or mineralize or freeze dry the sample, in order not to make sampling errors or even destroy the particle and its magnetization.

On the other hand, for preservation of tissue samples during storage or transport, it is no problem to fix the tissue. Tissues can immersed in fixation liquids, such as x% of Formalin. Also heparin can be added to blood samples to avoid blood coagulation.

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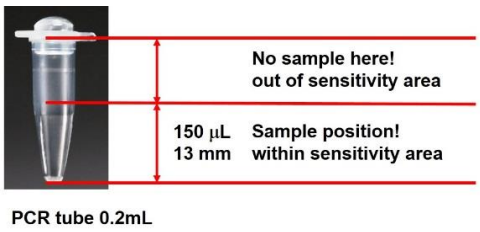
Sample volume

The tubes should be filled with maximum 150 µL sample.
Always keep total the total volume of all the samples constant over the full experiment to obtain consistent results, this is for all samples under test as well as for all calibration and background samples.

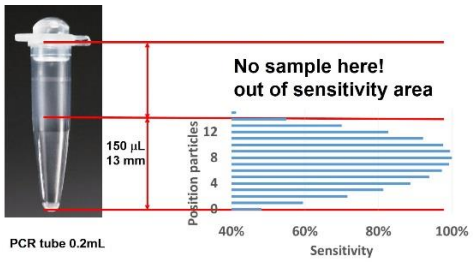
The tube must be filled starting from the bottom of the tip. The sample volume right under the cap will not be measured as it is out of the sensitivity area of the pEPR-system. Especially for large organ samples, the tissue must be cut in pieces, so that they fit into the tip of the PCR tube, and do not exceed the 150 µL volume (not in the cap of the tube as this will be out of the sensitivity area of the detection system).

For samples with particles or pre-labeled cells **homogeneously** dispersed in solution, tissue or blood:



Example:
Blood sample with Heparine
Total volume max 150 µL

For samples with particles or pre-labeled cells in **a well-defined section** of the tissue:

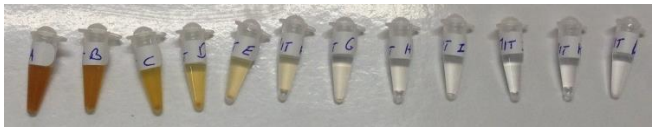


Example:
Aortic arch in x% EtOH
Total volume max 150 µL

When particles or cells are not homogeneously dispersed in the solution or tissue, it is important to have the section of the tissue with the particles in the area with maximal sensitivity:

Calibration measurement

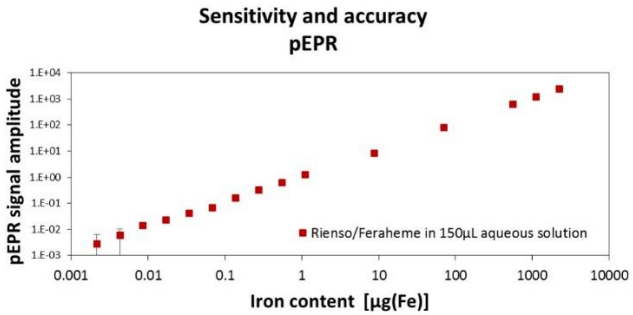
The most accurate calibration for quantification of the particles or labeled cells is obtained when a complete dilution curve is measured.



Particle quantification:
Typically, samples of particles in an aqueous solution with concentrations within the limits of the detection range of the pEPR-system are systematically measured to obtain a complete dilution or calibration curve.

Cell quantification:
Typically, samples of labeled cells in suspension and of the same batch as injected in the animal with concentrations within the limits of the detection range of the pEPR-system are systematically measured to obtain a complete dilution curve.

As an example the dilution or calibration curve for Rienso/Feraheme:



Background measurement

For the most accurate and correct analysis, especially at the very low particle concentrations, a background correction is made.
Background measurements consist of the pEPR measurement of a tissue sample of a control animal that is not exposed to the particles or the labeled cells, or of a blood sample taken just before particle or cell administration.
As of today, the pEPR measurements of blanc tissues, blood, pure water, HBS, PBS, agar agar, ... result in a signal below or at the detection limit, but a more systematic study is being performed.

Measurement result

The result obtained with pEPR is the amplitude of the pEPR response signal detected from the particles in resonance and the amplitude of the signal is measured in voltage [µV].

Particle quantification:

The **sensitivity** of the pEPR system for a particle is expressed as:

$$\text{Sensitivity } [\mu V/\mu g(Fe)] = \frac{\text{CALIBsample } [\mu V]}{\text{Specified amount of particles } [\mu g(Fe)]}$$

with the measured amplitude:
CALIBsample [µV]
this is the calibration sample with specified amount of particles [µg(Fe)]

The higher the sensitivity, the lower detection limit for that particle.
As a reference:
For Rienso/Feraheme the sensitivity is 29 µV/µg(Fe);
For Sinerem the sensitivity is 49 µV/µg(Fe).

Absolute quantification of particles is obtained according to the following formula:

$$\text{Sample } [\mu g(Fe)] = \frac{\text{Sample } [\mu V] - \text{BGRsample } [\mu V]}{\text{Sensitivity } [\mu V/\mu g(Fe)]}$$

with the measured amplitude for:
Sample [µV]: this is the sample under test, unknown amount of particles
BGRsample [µV]: this is a tissue, blood or pure solution sample without particles

Cell quantification:

The **sensitivity** of the pEPR system for the pre-labeled cells is expressed as:

$$\text{Sensitivity } [\mu V/\#\text{cells}] = \frac{\text{CALIBsample } [\mu V]}{\text{Specified amount of cells } [\#\text{cells}]}$$

with the measured amplitude:
CALIBsample [µV]
this is the calibration sample with specified amount of pre-labeled cells [#cells]

Absolute quantification of cells is obtained according to the following formula:

$$\text{Sample } [\#\text{cells}] = \frac{\text{Sample } [\mu V] - \text{BGRsample } [\mu V]}{\text{Sensitivity } [\mu V/\#\text{cells}]}$$

with the measured amplitude for:
Sample [µV]: the sample under test, unknown amount of pre-labeled cells
BGRsample [µV]: this is a tissue, blood or pure solution sample without pre-labeled cells