Samples and Measurement Protocol, and Shipping Guide for pEPR Measurements at Pepric

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.

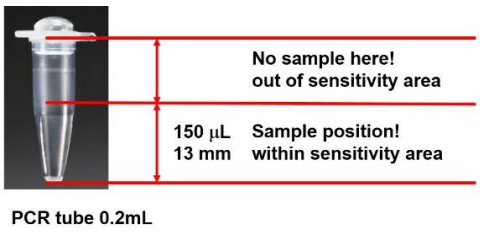
On the other hand, for preservation of tissue samples during storage or transport, it is no problem to fix the tissue. Tissues can immersed in fixation liquids, such as x% of Formalin. Also heparin can be added to blood samples to avoid blood coagulation.

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.

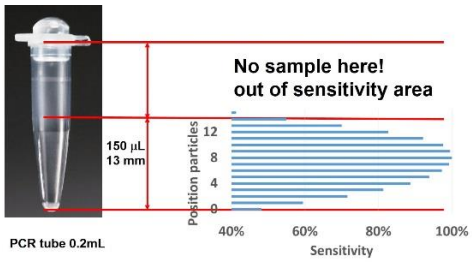
The tube must be filled starting from the bottom of the tip. The sample volume right under the cap will not be measured as it is out of the sensitivity area of the pEPR-system. Especially for large organ samples, the tissue must be cut in pieces, so that they fit into the tip of the PCR tube, and do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

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

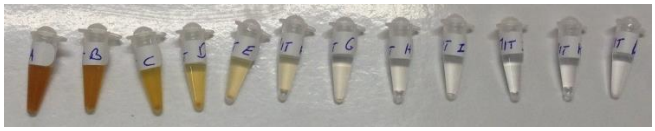


Example:
Aortic arch in x% EtOH
Total volume max 150 µL

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

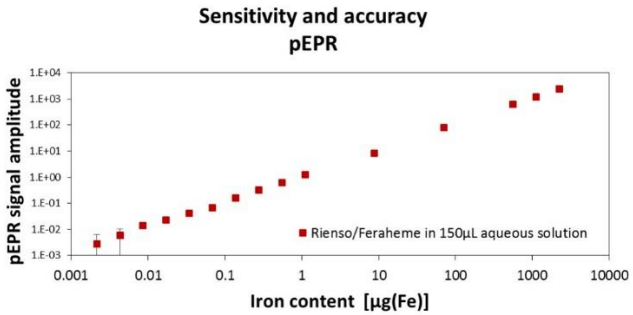
The most accurate calibration for quantification of the particles or labeled cells is obtained when a complete dilution curve is measured.



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

Sample measurements to preview the performance of the instrument

For a quick preview of the performance of instrument, you can send samples to Pepric for an sample analysis. To do so, please send a request to info@pepric.com, and after receiving an acknowledgement of the request, follow shipping guides as below.

Shipping guide of samples to Pepric for pEPR diagnosis when shipped from EU countries

- Samples must be packed in a leak-proof packaging:
  - Place PCR tubes in a sample container. Put the sample containers in a leak-proof packaging such as a zipper storage bag.
- The shipment must be properly packaged to prevent accidental opening of the package and breaking of the vials
  - If a fragile item such as a glass vial is being shipped, put the vial in a leak-proof packaging such as a zipper storage bag and wrap it with multiple layers of protective sheet such as bubble or foam sheet.
  - Fill empty space inside the outermost packaging box with shock absorbing material such as bubble wrap, crumpled paper, loose fill, or air cushions
- If the samples are from animals,
  - The following information must be stated on the packaging: name and location of sender and receiver, and the indication "For examination and diagnosis – do not open during transport"
  - The shipment must be accompanied by a certificate issued by the sender, stating the following:
    - The description of the material, and the animal species of origin
    - The category to which the material belongs defined by article 8, 9, and 10 of REGULATION (EC) No 1069/2009 (=category 1 material: parts of experimental animals)
    - Statement that the samples are from animals that did not show any symptoms of a disease communicable to humans or animals through that samples
    - The amount of material
    - The place of origin and the place of dispatch of the material
    - The name and address of the sender
    - The name and address of the addressee (Pepric).

Shipping guide of samples to Pepric for pEPR diagnosis when shipped from Non-EU countries

- If the samples are from animals, the following instructions should be followed in addition to the aforementioned instructions for samples from EU countries
  - the shipment must be additionally accompanied by a certificate issued by an **official veterinarian** (= veterinarian working for the competent authority in the third country) stating that the products come from animals that do not show any symptoms of a disease communicable to humans or animals through that product is required and is to be attached to the package;
  - prior to shipment, an import authorization by the Federal Agency for the Safety of the Food Chain (FASFC) of Belgium is required. Pepric will apply for an import authorization to FASFC. Please fill in the form, "Application form to obtain an authorization for imports from third countries of samples

of animal origin for research and diagnosis", in the appendix and send it to Pepric by email. The time from the submission of the form till an approval by FASFC varies from one to four weeks (typically, one to two weeks).
    - Pepric will initiate a shipping process so that a specialized courier that is allowed to ship biological samples by picking up your samples and deliver them to Pepric.
- If the shipment does not include any samples from animals, the same guideline applies as the aforementioned instructions for samples from the EU countries.

APENDIX

Application form for shipping samples of animal origin for research and diagnosis from countries outside of the EU to Pepric

Nature of samples for examination and / or diagnosis (including animal species and any treatment the products have undergone)	
Animal products are taken from:	☐ Bred[1] experimental[2] animals[3] ☐ Farmed animals[4] ☐ Wild animals[5] ☐ Pet animals[6] ☐ Aquatic animals[7] ☐ Other:
Do animal by-products contain pathogens, if so which ones?	
Quantity / number	
Origin (Name and address)	
Brief description of the planned examination and / or the planned diagnosis	

[1] 'bred animals' means animals specially bred for use in experiments in facilities approved by, or registered with, the authority;
[2] 'experimental animals' means animals used or to be used in experiments; 'experiment' means any use of an animal for experimental or other scientific purposes which may cause it pain, suffering, distress or lasting harm, including any course of action intended, or liable, to result in the birth of an animal in any such condition, but excluding the least painful methods accepted in modern practice (i.e. 'humane' methods) of killing or marking an animal; an experiment starts when an animal is first prepared for use and ends when no further observations are to be made for that experiment; the elimination of pain, suffering, distress or lasting harm by the successful use of anesthesia or analgesia or other methods does not place the use of an animal outside the scope of this definition. Non experimental, agricultural or clinical veterinary practices are excluded;
[3] 'animal' unless otherwise qualified, means any live non-human vertebrate, including free-living larval and/or reproducing larval forms, but excluding foetal or embryonic forms;
[4] 'farmed animal' is any animal that is kept, fattened or bred by humans and used for the production of food, wool, fur, feathers, hides and skins or any other product obtained from animals or for other farming purposes
[5] 'wild animal' means any animal not kept by humans;
[6] 'pet animal' means any animal belonging to species normally nourished and kept but not consumed, by humans for purposes other than farming;
[7] 'aquatic animals' means (i) fish belonging to the superclass Agnatha and to the classes Chondrichthyes and Osteichthyes; (ii) mollusc belonging to the Phylum Mollusca; (iii) crustacean belonging to the Subphylum Crustacea;