

I have been using your ATP probe on mouse brain slices and have a few quick questions. I have been placing the very tip of the probe so that it is just touching the surface of the brain slice. Is this optimal? Or would I get better results if I bent the tip of the probe so that the entire enzyme coated area lay flat across the surface? Would bending the tip of the probe ruin it?

Sensor placement depends on application -in general we recommend trying to maximize the contact of the sensor surface area with the sites of release in the tissue.

This means either pushing the sensor through the entire depth of the slice, or as you suggest bending the sensor so that you can lay it flat on the slice (if you confine yourself to surface measurements). Whether you use surface or within-slice measurements depends very much on where you think the ATP is coming from. We have used both methods e.g.

Wall and Dale 2007 J Physiol;

Frenguelli et al 2007 J Neurochem;

Gourine et al Nature 2005.

Bending of the tip is possible and will not ruin the probe. It is best to do this using a binocular microscope so that you see what you are doing. Using a fine pair of forceps, bend the white polymer coated part of the sensor. Avoid touching the exposed active (enzyme coated) part of the sensor (this will ruin it) -but with care you can bend the white part sufficiently close to the active area to get the correct angle so the sensors can be laid flat on the slice.

We are interested in measuring acetylcholine in behaving animals. I understand that you do offer Ach probes.

To measure ACH, as opposed to choline, you need to use two sensors -one for ACH (that also detects choline) and one for just choline -then subtract recordings of the second from the first to get net ACH.

I'm interested in measuring adenosine and ATP level in mouse or rat brain in vivo. I'm wondering if it's possible I can measure any of them in a freely moving animal with your product. If you have any experience, data, or pictures, please let me see them. And what are the detection limits of them?

The sarissaprobe products are not really suited to measurements from freely moving animals. They have been used extensively in vivo in anesthetized animals and the publications list on the website gives examples of this.

We have made custom sensors for freely moving animals (Gourine et al, 2007, J Physiol 585). However we recommend that you consider using Pinnacle Technologies products -these are specifically designed for use in freely moving animals. We have recently arranged a collaboration agreement with Pinnacle where by we can use the Sarissa coating technology to provide ATP and adenosine biosensors on their assemblies.

I have a few technical questions regarding the biosensors - maybe you can help me get the answers from the right persons (I'm a little confused about your email addresses).

Can you confirm that standard products from Sigma ([A9251](#) Adenosine, [I4125](#) Inosine, [A7699](#) ATP-disodium salt, [G5516](#) Glycerol) would be appropriate for the preparation of the sensors.

Do you have any guidelines regarding the handling and storage of buffers and standards after preparation? Do you prepare these on a daily basis or do you store them at -20 or 5 C, and for how long?

Your chemicals from Sigma should be fine for our sensors. Although we use ATP rather than ATP-disodium salt.

As those testing solutions degrade in a warm environment, we suggest you make up a 10mM stock solution daily (preferably keep on ice on the day), then make up the final 10uM testing solution. And re-make the solution from the stock if it's been in warm condition for long hours.

Phosphate buffer (with glycerol) should be fine under room temperature for months. But if you intend to store the sensors in the buffer between the experiments, you need to keep them in the fridge!

Adenosine and inosine stock solutions at 10mM can be made up in pure H₂O and stored in fridge for several days. Dilute these stocks into saline as required (we routinely use a 10uM test solution). Note that Ado is not particularly soluble in water so some mild heating may be necessary to get it to dissolve initially (and if the stock has been in the fridge for a few days Ado tends to come out of solution). ATP stock solutions (10mM in pure H₂O) should be made fresh each day and stored on ice. If you heat your salines to near physiological temp do not heat the ATP test solution (it breaks down and is transformed into an electroactive substance quite quickly) -either keep the ATP test solution at room temp (fine for several hours) or if heating make your ATP test solution fresh for each calibration. Again we habitually use a 10uM test solution of ATP for calibration.