SPARCL™ Assay FAQs

1. How long do the assays take to set up and run?
    - The homogeneous assays in which acridan and HRP conjugates are mixed directly with sample in the SPARCL™ plate require one 30-minute incubation[1] plus approximately 15-minutes set-up time.
    - The capture assays in which the antibody target (i.e., KLH) is immobilized on the plate require two 30-minute incubations plus 15-minutes set-up time.

2. Can plasma be used in the assays?
    - EDTA and citrate plasma cannot be used in the homogeneous assays unless samples are diluted at least 100-fold with the kit diluent. EDTA and citrate inhibit HRP.
    - Heparin plasma can be used.

3. Can I substitute my own dilution buffer(s)?
    - No. We have identified suitable diluents for each assay.

4. I don't have a -70 or -80^{o}C freezer. Can I still use the SPARCL™ assays?
    - Yes. You may store the conjugates at -20^{o}C for 1-2 weeks. However, when stored at -20^{o}C, the acridan conjugate will slowly degrade, leading to lower RLU values. The lower RLU values do not affect assay performance. If you don't have a -70 or -80^{o}C freezer, we suggest that you plan on ordering the kit(s) a few days before they are needed.

5. Can I re-freeze the unused conjugate stocks?
    - Yes. Shortly after use, re-freeze unused conjugate. Both HRP and acridan conjugates withstand multiple freeze-thaws.

6. Are the assays susceptible to a prozone, or hook effect?
    - The homogeneous assays in which acridan and HRP conjugates are mixed directly with sample in the SPARCL™ plate are susceptible to a hook effect at high concentrations of biomarker. However, we have designed the assays such that, if you use our suggested dilutions, this possibility is minimized.
    - If you are concerned that a hook effect is causing false-low values, test the samples at several different dilutions and check for linearity with dilution.

7. Can I use other plates for the assay?
    - No. We have coated the white SPARCL™ plates with a reagent that reduces background signal. If you use non-coated coated plates very high background values will result, invalidating the assay.

8. Must I run a standard curve with every assay?
    - Yes.

9. Do I need to add background reducer?
    - No. The SPARCL™ plate has been pre-coated with a background reducing agent.

[1] A few SPARCL assays developed by LDI use a 45-minute incubation.

10. My Luminometer offers a variety of options for data calculations, i.e., slope, time to threshold, time to max, sum, etc. - which should I use?
    - **Always use the sum of RLU values.**
    - We recommend using the sum of RLU values between 100 and 980 ms. This eliminates luminescence spikes/artefacts that sometimes occur during the first 100 ms after trigger injection.
11. I find high variability within replicates. Why?
    - Imprecise pipetting is the leading cause of variability. Rather than using a 1.25 ml electronic pipette to dispense multiple 25.0 or 50.0 µl aliquots of conjugate mix to the wells, use a 250 µl electronic pipettor instead. You can also pipet individual aliquots using a single channel 100 µl pipettor.
    - When pipetting samples into the wells do not "blow-out" the conjugate mix, standards or samples. Bubbles caused by this action will cause variability.
    - Slow addition of standards and samples to the plate can also lead to high variability (see the next FAQ).
    - Proper pipetting technique is critical to quality results in SPARCL and all immunoassays. Training in the proper use of pipets is strongly encouraged.

12. How quickly should standards and samples be added to the SPARCL™ plate?
    - Within 3 minutes. The relatively short incubation time (30 min) does not allow the assay to reach equilibrium.
    - We prepare standards and samples in a clear polystyrene plate using the layout planned for the SPARCL™ plate. This allows standards and samples to be added to the SPARCL™ plate within 1-2 minutes using a multichannel pipettor.

13. What happens if I forgot to prime the luminometer with trigger solution before running my plate through the luminometer?
    - When trigger solution is not injected into wells of the SPARCL™ plate, the chemical reaction that generates light does not occur. As a result, there will be no signal generated and no data available from those wells.
    - If you injected air into the wells by mistake, prime the luminometer and re-run the plate.

14. Can I read my plate twice?
    - No. SPARCL is flash luminescence. Once the trigger solution is added, light is emitted and the acridan conjugate is exhausted.

15. Do I need to protect my reagents from light?
    - You may set up and run your assay in normal laboratory or room light. You should not expose your reagents to direct sunlight or other sources of intense light.