To prepare the ROC analysis with obex, follow the instructions below. If you are using the provided data (RT-QuIC Obex Input Data.xlsx), please skip to step 4. If you are providing your own data, please follow the same format.

1. In your BMG analysis software, click “Table View”. In the top right in the “Cycle” tab, click “All”
2. Click Excel Report – Export displayed table to Excel.
3. Prepare the template.
   1. Open the file RT-QuIC Obex Input Data.xlsx
      1. Each row corresponds to the fluorescence data of a single replicate
         1. Column A: A descriptor for the replicate
         2. Column B: A descriptor for the replicate
         3. Column C: Concentration (w/v) of the control sample
         4. Column D: Sample Type Currently Tested
         5. Column E: Tissue
         6. Column F: Average baseline reading of plate
         7. Column G: Sample standard deviation of plate
         8. Column H: ELISA/IHC/Bioassay/WB Status (Positive/Negative)
         9. Column I: Signal threshold being used (e.g. baseline + 10 sample standard deviations.
         10. Column J: Leave empty
   2. Copy and paste the RFU data outputted into Excel from the BMG software into the RT-QuIC Obex Input Data.xlsx template. The first RFU reading should align with column K.
4. Open the file “Obex Model Generation.R”. Run the script from lines 1-4. This will install the necessary packages. After the packages are downloaded, restart the R environment (Click “Session”, then “Clear workspace”, then “Yes”).
5. Run the script from lines 1-138. The script will then suggest which dilutions are valid in the “Dilution p Values” sheet in the “RT-QuIC Obex Output Data.xlsx” file. Adjust lines 141-142 to include the dilutions of interest.
6. Run the remainder of the script. Once the script has completed running (~20-25 min, depending on single-thread computing power), type “ctCutoff” into the console to get the optimized Ct cut-off. Our ctCutoff was 33 hours.
7. To determine the ideal assay duration with MPR, open the “MPR Threshold Data” sheet in the “RT-QuIC Obex Output Data.xlsx” file. For our analysis, we decided the ideal assay duration was when the rate (i.e. dAUC/dt) consistently dropped below 0.01. This corresponded to an assay duration of 102 cycles (29.3 hours) with a TMPR of 3.36.

We have now determined the ideal assay duration with Tstdev, and the ideal assay duration with TMPR and the optimized TMPR.

If interested, the same analysis can be completed with a different tissue. We have attached the “RLN Model Generation.R” script and the “RT-QuIC RLN Input Data.xlsx” data file.