



# Rigaku

## INSTRUCTION SHEET

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### TIME Detergent Screen 1008653

The TIME (Total Integral Membrane-Protein Extraction) detergent screen aids the identification of the detergent reagent that successfully extracts a membrane protein from a membrane preparation. Further analysis can be carried out by ultracentrifugation, Fluorescence detection SEC-HPLC.

Membrane protein extraction trials are carried out in the following way:

1. Remove seal from plate *after spinning it to collect all liquid within the well.*
2. Mix 125  $\mu$ L of membrane preparation with the contents of each well in a fresh 96-well plate. Membranes should be prepared at a concentration that allows for good signal: noise in the analytical assay of your choice. *For instance: 20mg/mL total protein concentration is suitable for standard FSEC experiments.*
3. Incubate the mixture *on a rotator* to allow for protein extraction to occur. Different incubation times and temperatures will influence extraction efficiency; however typically 1 hour at 4°C can serve as a good starting point for extraction optimization.
4. Separate extracted material by ultracentrifugation at  $>100,000 \times g$  for 20 minutes using a small volume rotor or filtration using a 0.22  $\mu$ m filter. Use either the supernatant (if using ultracentrifugation) or the flow through (if using filtration) for step 5.
5. Analyze samples for protein activity and/or for solution behavior via analytical fluorescence detection SEC-HPLC and/or ligand binding experiment.
6. Long term storage -80 C