Brief Protocol for performing Prozomix kREDy-to-go plate assay screens

[Yuyin Qi, 15-10-12] – for any further information, please email: yuyin.qi@prozomix.com

1. Blank plate:

(a) Aliquot 10 mL INT solution (0.25 mg/mL) into a reagent reservoir.

(b) Using the microplate sealing film cross-hairs as a guide, carefully add 0.10 mL of the INT solution to each well using a multi-channel (or normal) pipette and mix carefully to reconstitute the freeze-dried enzymes. Change tips between additions, to avoid enzyme cross-talk.

(c) Incubate at ambient temperature in the dark for up to 24 h (though results may be seen as quickly as 15 min).

2. Assay plate:

(a) Aliquot 10 mL INT solution (0.25 mg/mL) into a reagent reservoir.

(b) Add 0.05 mL (or 0.05 g if a solid) target alcohol to the INT solution and stir until fully dissolved.

(c) Using the microplate sealing film cross-hairs as a guide, carefully add 0.10 mL of the INT/alcohol solution to each well using a multi-channel (or normal) pipette and mix carefully to reconstitute the freeze-dried enzymes. Change tips between additions, to avoid enzyme cross-talk.

(d) Incubate at ambient temperature in the dark for up to 24 h (though results may be seen as quickly as 15 min).

NOTE: (i) only 1 blank plate is required in the case of multiple assay plates

(ii) stack the plates together to ensure they all incubate at the same temperature

(iii) the plates can be inspected manually by for instance holding above a white sheet of paper, or alternatively, they may be analysed using a microplate reader at 492 nm. If it is elected to read on a microplate reader, best results will be obtained by first mixing the plates on a plate shaker, then pulse centrifuging them, followed by removal of the cross-hair films.

(iv) **OPTIONAL** - confirm interesting results by performing another assay plate. This can be useful, as infrequently sporadic alcohol contamination of pipette tips or other plastic s used can lead to false positives (or negatives, if appearing on the blank plate). As kREDy-to-go plates are provided free of charge, it is thus recommended that interesting results be confirmed before other more time consuming/expensive analyses, such as chiral HPLC or chiral GC, be performed.

3. Typical results (cell “A1” is top left, cell “H12” is bottom right):