**Plate layout**

- Stability test 0 hour

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NAC | A | | | | | | | | | | | | |
| | B | | | | | | | | | | | | |
| | C | | | | | ST(0) | ST(0) | ST(0) | | | | | |
| | D | | | | | | | | | | | | |
| NAL | E | | | | | | | | | | | | |
| | F | | | | | ST(0) | ST(0) | ST(0) | | | | | |
| | G | | | | | | | | | | | | |
| | H | | | | | | | | | | | | |

- For five test chemicals and one solvent (Acetonitrile)

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NAC | A | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | RC-A | RC-B | RC-B | RC-C | PC | std.1 | std.5 |
| | B | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | RC-A | RC-B | RC-B | RC-C | PC | std.2 | std.6 |
| | C | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | RC-A | RC-B | RC-B | RC-C | PC | std.3 | std.7 |
| | D | CC-1 | CC-2 | CC-3 | CC-4 | CC-5 | ST(24) | ST(24) | ST(24) | - | - | std.4 | - |
| NAL | E | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | RC-A | RC-B | RC-B | RC-C | PC | std.1 | std.5 |
| | F | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | RC-A | RC-B | RC-B | RC-C | PC | std.2 | std.6 |
| | G | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | RC-A | RC-B | RC-B | RC-C | PC | std.3 | std.7 |
| | H | CC-1 | CC-2 | CC-3 | CC-4 | CC-5 | ST(24) | ST(24) | ST(24) | - | - | std.4 | - |

- For 17 test chemicals and one solvent (Acetonitrile)
  First plate

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NAC | A | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Sample 11 | Sample 12 |
| | B | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Sample 11 | Sample 12 |
| | C | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Sample 11 | Sample 12 |
| | D | CC-1 | CC-2 | CC-3 | CC-4 | CC-5 | CC-6 | CC-7 | CC-8 | CC-9 | CC-10 | CC-11 | CC-12 |
| NAL | E | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Sample 11 | Sample 12 |
| | F | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Sample 11 | Sample 12 |
| | G | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | Sample 11 | Sample 12 |
| | H | CC-1 | CC-2 | CC-3 | CC-4 | CC-5 | CC-6 | CC-7 | CC-8 | CC-9 | CC-10 | CC-11 | CC-12 |

  Second plate

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NAC | A | Sample 13 | Sample 14 | Sample 15 | Sample 16 | Sample 17 | RC-A | RC-B | RC-B | RC-C | PC | std.1 | std.5 |
| | B | Sample 13 | Sample 14 | Sample 15 | Sample 16 | Sample 17 | RC-A | RC-B | RC-B | RC-C | PC | std.2 | std.6 |
| | C | Sample 13 | Sample 14 | Sample 15 | Sample 16 | Sample 17 | RC-A | RC-B | RC-B | RC-C | PC | std.3 | std.7 |
| | D | CC-13 | CC-14 | CC-15 | CC-16 | CC-17 | ST(24) | ST(24) | ST(24) | - | - | std.4 | - |
| NAL | E | Sample 13 | Sample 14 | Sample 15 | Sample 16 | Sample 17 | RC-A | RC-B | RC-B | RC-C | PC | std.1 | std.5 |
| | F | Sample 13 | Sample 14 | Sample 15 | Sample 16 | Sample 17 | RC-A | RC-B | RC-B | RC-C | PC | std.2 | std.6 |
| | G | Sample 13 | Sample 14 | Sample 15 | Sample 16 | Sample 17 | RC-A | RC-B | RC-B | RC-C | PC | std.3 | std.7 |
| | H | CC-13 | CC-14 | CC-15 | CC-16 | CC-17 | ST(24) | ST(24) | ST(24) | - | - | std.4 | - |

・For 10 test chemicals and two solvent

First plate

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NAC | A | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | – | – |
| | B | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | – | – |
| | C | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | – | – |
| | D | CC-1 | CC-2 | CC-3 | CC-4 | CC-5 | CC-6 | CC-7 | CC-8 | CC-9 | CC-10 | – | – |
| NAL | E | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | – | – |
| | F | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | – | – |
| | G | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 | Sample 7 | Sample 8 | Sample 9 | Sample 10 | – | – |
| | H | CC-1 | CC-2 | CC-3 | CC-4 | CC-5 | CC-6 | CC-7 | CC-8 | CC-9 | CC-10 | – | – |

Second plate

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NAC | A | RC-A | RC-B | RC-B | RC-C Solvent 1 | RC-C Solvent 2 | PC | – | – | – | – | std.1 | std.5 |
| | B | RC-A | RC-B | RC-B | RC-C Solvent 1 | RC-C Solvent 2 | PC | – | – | – | – | std.2 | std.6 |
| | C | RC-A | RC-B | RC-B | RC-C Solvent 1 | RC-C Solvent 2 | PC | – | – | – | – | std.3 | std.7 |
| | D | – | – | – | – | – | ST(24) | ST(24) | ST(24) | – | – | std.4 | – |
| NAL | E | RC-A | RC-B | RC-B | RC-C Solvent 1 | RC-C Solvent 2 | PC | – | – | – | – | std.1 | std.5 |
| | F | RC-A | RC-B | RC-B | RC-C Solvent 1 | RC-C Solvent 2 | PC | – | – | – | – | std.2 | std.6 |
| | G | RC-A | RC-B | RC-B | RC-C Solvent 1 | RC-C Solvent 2 | PC | – | – | – | – | std.3 | std.7 |
| | H | – | – | – | – | – | ST(24) | ST(24) | ST(24) | – | – | std.4 | – |

**Sample**

Reaction solution of NAC and NAL with test chemical. Evaluate reactivity of NAC or NAL with the test chemical at a ratio of 1: 200 for the NAC/NAL with the test chemical.

Composition…Test Chemical Solution (4 mM): NAC Solution / NAL Solution (6.667 µM) = 50 µL: 150 µL

**PC (Positive Control)**

Composition…4 mM Phenylacetaldehyde/ Squaric Acid Diethyl Ester Acetonitrile Solution: NAC Solution or NAL Solution (6.667 µM)=50 µL: 150 µL

**RC (Reference Control)**

**RC-A**

Control for verifying validity of the HPLC system. Reference Control A is used to verify concentration of NAC and NAL from each calibration curve after addition of acetonitrile rather than test chemical.

Composition…Acetonitrile: NAC Solution or NAL Solution (6.667 µM)=50 µL: 150 µL

**RC-B**

Control for verifying stability of reaction solution under analysis. Reference Control B is used to verify variability (CV) of each three NAC/NAL peak areas in the solution after addition of acetonitrile rather than test chemical at the start of analysis and at the end of analysis.

Composition…Acetonitrile: NAC Solution or NAL Solution (6.667 µM) = 50 µL: 150 µL

**RC-C**

Control for calculating NAC and NAL depletion of each test chemical solution. To calculate depletion of NAC and NAL, measure three Reference Controls C after addition of solvent instead of test chemical. Prepare reference Control C for all solvents used to dissolve the test chemicals.

Composition…Acetonitrile* and solvent used for dissolution of test chemical: NAC Solution or NAL Solution (6.667 µM)=50 µL: 150 µL

*Be sure to prepare acetonitrile addition RC-C.

**CC (Co-elution Control)**

Control for verifying whether or not test chemicals co-elute with NAC or NAL. The Co-elution Control is used to verify absorbance at 281 nm and whether retention time of test chemical is equal to that of NAC or NAL.

Composition…Test Chemical Solution (4 mM): NAC Buffer or NAL Buffer=50 µL: 150 µL

**ST (Stability Test)**

Test for NAC solution and NAL solution stability. Stability test is tested to the residual ratio of NAC solution and NAL solution at preparation (ST0) and 24 hours after preparation (ST24).

Composition…NAC Solution or NAL Solution (6.667µM): Acetonitrile=150 µL: 50 µL