**iPOND Proteomics Susanne/CA**

**Samples:**

* xxx cells (100 million per sample, 10 plates per condition), iPOND comparing chromatin restoration (0-2h) upon transcription inhibition
* IP and Input (LI), each contain control and Nascent/1h/2h for DMSO/1 uM TPL/ 50 uM DRB, 3 bio-replicates (SB45/46/47)
* 60 samples in total

|  |  |  |  |
| --- | --- | --- | --- |
| Sample Batch | Samples Type | Name | Starting Volume/uL |
| SB45 | Input (LI) | Negative Control (no EdU) | 50 |
| SB45 | Input (LI) | DMSO-Nascent | 50 |
| SB45 | Input (LI) | DMSO-1h | 50 |
| SB45 | Input (LI) | DMSO-2h | 50 |
| SB45 | Input (LI) | 1uM TPL-Nascent | 50 |
| SB45 | Input (LI) | 1uM TPL-1h | 50 |
| SB45 | Input (LI) | 1uM TPL-2h | 50 |
| SB45 | Input (LI) | 50uM DRB-Nascent | 50 |
| SB45 | Input (LI) | 50uM DRB-1h | 50 |
| SB45 | Input (LI) | 50uM DRB-2h | 50 |
| SB46 | Input (LI) | Negative Control (no EdU) | 50 |
| SB46 | Input (LI) | DMSO-Nascent | 50 |
| SB46 | Input (LI) | DMSO-1h | 50 |
| SB46 | Input (LI) | DMSO-2h | 50 |
| SB46 | Input (LI) | 1uM TPL-Nascent | 50 |
| SB46 | Input (LI) | 1uM TPL-1h | 50 |
| SB46 | Input (LI) | 1uM TPL-2h | 50 |
| SB46 | Input (LI) | 50uM DRB-Nascent | 50 |
| SB46 | Input (LI) | 50uM DRB-1h | 50 |
| SB46 | Input (LI) | 50uM DRB-2h | 50 |
| SB47 | Input (LI) | Negative Control (no EdU) | 50 |
| SB47 | Input (LI) | DMSO-Nascent | 50 |
| SB47 | Input (LI) | DMSO-1h | 50 |
| SB47 | Input (LI) | DMSO-2h | 50 |
| SB47 | Input (LI) | 1uM TPL-Nascent | 50 |
| SB47 | Input (LI) | 1uM TPL-1h | 50 |
| SB47 | Input (LI) | 1uM TPL-2h | 50 |
| SB47 | Input (LI) | 50uM DRB-Nascent | 50 |
| SB47 | Input (LI) | 50uM DRB-1h | 50 |
| SB47 | Input (LI) | 50uM DRB-2h | 50 |
| SB45 | IP | Negative Control (no EdU) | 70 |
| SB45 | IP | DMSO-Nascent | 70 |
| SB45 | IP | DMSO-1h | 70 |
| SB45 | IP | DMSO-2h | 70 |
| SB45 | IP | 1uM TPL-Nascent | 70 |
| SB45 | IP | 1uM TPL-1h | 70 |
| SB45 | IP | 1uM TPL-2h | 70 |
| SB45 | IP | 50uM DRB-Nascent | 70 |
| SB45 | IP | 50uM DRB-1h | 70 |
| SB45 | IP | 50uM DRB-2h | 70 |
| SB46 | IP | Negative Control (no EdU) | 70 |
| SB46 | IP | DMSO-Nascent | 70 |
| SB46 | IP | DMSO-1h | 70 |
| SB46 | IP | DMSO-2h | 70 |
| SB46 | IP | 1uM TPL-Nascent | 70 |
| SB46 | IP | 1uM TPL-1h | 70 |
| SB46 | IP | 1uM TPL-2h | 70 |
| SB46 | IP | 50uM DRB-Nascent | 70 |
| SB46 | IP | 50uM DRB-1h | 70 |
| SB46 | IP | 50uM DRB-2h | 70 |
| SB47 | IP | Negative Control (no EdU) | 70 |
| SB47 | IP | DMSO-Nascent | 70 |
| SB47 | IP | DMSO-1h | 70 |
| SB47 | IP | DMSO-2h | 70 |
| SB47 | IP | 1uM TPL-Nascent | 70 |
| SB47 | IP | 1uM TPL-1h | 70 |
| SB47 | IP | 1uM TPL-2h | 70 |
| SB47 | IP | 50uM DRB-Nascent | 70 |
| SB47 | IP | 50uM DRB-1h | 70 |
| SB47 | IP | 50uM DRB-2h | 70 |

**Sample preparation**

* All LI samples (50 uL) are in 0.5% SDS in 25 mM Tris, pH 8 + 2x LSB (Laemmli sample buffer, 100 mM Tris-HCl PH 6.8, 200 mM DTT, 4% SDS, 20% glycerol)
* All IP samples (70 uL) are in 2x LSB (Laemmli sample buffer, 100 mM Tris-HCl PH 6.8, 200 mM DTT, 4% SDS, 20% glycerol)
* Samples were boiled at 95ºC for 10 min
* Sonicate samples in a bath sonicator (Bioruptor) for 10 cycles (30 s on, 30 s off)
* Reduce reversibly oxidized cysteines with 10 mM TCEP (30 min, RT, 1000 rpm)
  + Add 1 µL 500 mM TCEP in LI samples
  + Add 1.4 µL 500 mM TCEP in IP samples
* Alkylate free thiols with 400 mM IAA (1 h, RT, 1000 rpm, dark)
  + Add 70 µL 800 mM IAA in both LI and IP samples
* Measure sample volumes:
  + Input, only use 120 µL
  + IP, only use 140 µL
* EZQ: Measure protein concentration (Y:\Plate reader\20210519)

Digestion with SP3 method (**s**ingle-**p**ot **s**olid-**p**hase-enhanced **s**ample **p**reparation):

* Sample transferred into 2 mL tubes (Input: 120 µL, IP: 140 µL)
* Add xx uL 100 mM Tris-HCl (pH 8.0) and xx µL SP3 (10:1, beads: protein) magnetic beads (20 µg/µL; *Sera-Mag SpeedBead Carboxylate-Modified magnetic particles (hydrophilic/hydrophobic 1:1) (GE Healthcare Life Sciences)*)
* Add xx µL 100% ACN (to f.c. 70%)
* Incubate for 10 min (RT, 1000 rpm)
* Mount tubes on magnetic rack (2 min)
* Remove supernatant
* Wash beads, while tube is on rack (2x 70% EtOH, 1x ACN, 1 mL each)
* Remove tubes from magnetic rack
* Redissolve beads in 80 µL digest buffer (50 mM TEAB) through shaking (few minutes, 1000 rpm)
* Digestion with LysC 4 h (1:100 enzym:protein ratio, 37 ºC, 1000 rpm)
  + IP: xx µg
  + Input: xx µg
* Digestion with trypsin overnight (1:50 enzym:protein ratio, 37 ºC, 1000 rpm)
  + IP: xx µg
  + Input: xx µg
* After digestion acidify with 9 µL 10 % formic acid (f.c. 1%)
* Add 1900 µL 100% ACN (f.c. 95%)
* Incubate for 10 min (RT, 1000 rpm)
* Centrifuge (15 s, 1000 g)
* Mount tubes on magnetic rack (2 min)
* Remove supernatant
* Wash beads (ACN, 1.5 mL), while tube is on rack
* Redissolve beads in 100 µL 2% DMSO (twice, 200 uL in total)
* Incubate for 10 min (RT, 1000 rpm) and mount tubes on magnetic rack (2 min)
* Transfer supernatant (= peptides) into new tube (0.5 mL)
* Quantify peptides with Pierce™ Quantitative Colorimetric Peptide Assay (Y:\Plate reader\20210521)

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample Batch | Samples Type | Name | Starting Volume/uL | Concentration (ug/uL) | Protein/ug | Theoretical Beads (ug) | Actural Beads (ug) | Beads (uL) | Add 100 mM Tris-HCl (pH 8)/uL | ACN (uL) | Lys-C (ug) | Trypsin (ug) | TMT labelling/uL | LOT | TMT Tag |
| SB45 | Input (LI) | Negative Control (no EdU) | 50 | 0.359660674 | 43.159281 | 431.5928086 | 431.5928086 | 21.57964 | 58.4 | 800 | 0.4 | 0.9 | 50 | UL298812 | 126 |
| SB45 | Input (LI) | DMSO-Nascent | 50 | 0.285691136 | 34.282936 | 342.8293629 | 342.8293629 | 17.141468 | 62.9 | 800 | 0.3 | 0.7 | 50.0 | UL298812 | 127N |
| SB45 | Input (LI) | DMSO-1h | 50 | 0.24613824 | 29.536589 | 295.3658879 | 295.3658879 | 14.768294 | 65.2 | 800 | 0.3 | 0.6 | 50.0 | UL298812 | 127C |
| SB45 | Input (LI) | DMSO-2h | 50 | 0.272423473 | 32.690817 | 326.9081676 | 326.9081676 | 16.345408 | 63.7 | 800 | 0.3 | 0.7 | 50.0 | UL298812 | 128N |
| SB45 | Input (LI) | 1uM TPL-Nascent | 50 | 0.28425438 | 34.110526 | 341.1052558 | 341.1052558 | 17.055263 | 62.9 | 800 | 0.3 | 0.7 | 36.8 | UL298812 | 128C |
| SB45 | Input (LI) | 1uM TPL-1h | 50 | 0.217606002 | 26.11272 | 261.1272027 | 261.1272027 | 13.05636 | 66.9 | 800 | 0.3 | 0.5 | 50.0 | UL298812 | 129N |
| SB45 | Input (LI) | 1uM TPL-2h | 50 | 0.283408404 | 34.009008 | 340.0900843 | 340.0900843 | 17.004504 | 63.0 | 800 | 0.3 | 0.7 | 35.5 | UL298812 | 129C |
| SB45 | Input (LI) | 50uM DRB-Nascent | 50 | 0.353163798 | 42.379656 | 423.7965574 | 423.7965574 | 21.189828 | 58.8 | 800 | 0.4 | 0.8 | 45.6 | UL298812 | 130N |
| SB45 | Input (LI) | 50uM DRB-1h | 50 | 0.268664429 | 32.239732 | 322.3973153 | 322.3973153 | 16.119866 | 63.9 | 800 | 0.3 | 0.6 | 47.6 | UL298812 | 130C |
| SB45 | Input (LI) | 50uM DRB-2h | 50 | 0.228038433 | 27.364612 | 273.6461191 | 273.6461191 | 13.682306 | 66.3 | 800 | 0.3 | 0.5 | 50.0 | UL298812 | 131 |
| SB46 | Input (LI) | Negative Control (no EdU) | 50 | 0.614751205 | 73.770145 | 737.7014457 | 737.7014457 | 36.885072 | 43.1 | 800 | 0.7 | 1.5 | 50.0 | VH306773 | 126 |
| SB46 | Input (LI) | DMSO-Nascent | 50 | 0.456056449 | 54.726774 | 547.2677393 | 547.2677393 | 27.363387 | 52.6 | 800 | 0.5 | 1.1 | 50.0 | VH306773 | 127N |
| SB46 | Input (LI) | DMSO-1h | 50 | 0.465884064 | 55.906088 | 559.0608771 | 559.0608771 | 27.953044 | 52.0 | 800 | 0.6 | 1.1 | 50.0 | VH306773 | 127C |
| SB46 | Input (LI) | DMSO-2h | 50 | 0.500248817 | 60.029858 | 600.2985798 | 600.2985798 | 30.014929 | 50.0 | 800 | 0.6 | 1.2 | 50.0 | VH306773 | 128N |
| SB46 | Input (LI) | 1uM TPL-Nascent | 50 | 0.439859132 | 52.783096 | 527.8309579 | 527.8309579 | 26.391548 | 53.6 | 800 | 0.5 | 1.1 | 45.0 | VH306773 | 128C |
| SB46 | Input (LI) | 1uM TPL-1h | 50 | 0.376276939 | 45.153233 | 451.532327 | 451.532327 | 22.576616 | 57.4 | 800 | 0.5 | 0.9 | 50.0 | VH306773 | 129N |
| SB46 | Input (LI) | 1uM TPL-2h | 50 | 0.487014329 | 58.44172 | 584.4171951 | 584.4171951 | 29.22086 | 50.8 | 800 | 0.6 | 1.2 | 44.1 | VH306773 | 129C |
| SB46 | Input (LI) | 50uM DRB-Nascent | 50 | 0.428163479 | 51.379617 | 513.7961746 | 513.7961746 | 25.689809 | 54.3 | 800 | 0.5 | 1.0 | 50.0 | VH306773 | 130N |
| SB46 | Input (LI) | 50uM DRB-1h | 50 | 0.315573363 | 37.868804 | 378.6880351 | 378.6880351 | 18.934402 | 61.1 | 800 | 0.4 | 0.8 | 48.7 | VH306773 | 130C |
| SB46 | Input (LI) | 50uM DRB-2h | 50 | 0.471346544 | 56.561585 | 565.6158528 | 565.6158528 | 28.280793 | 51.7 | 800 | 0.6 | 1.1 | 44.1 | VH306773 | 131 |
| SB47 | Input (LI) | Negative Control (no EdU) | 50 | 0.496517419 | 59.58209 | 595.8209031 | 595.8209031 | 29.791045 | 50.2 | 800 | 0.6 | 1.2 | 50.0 | VH306773 | 126 |
| SB47 | Input (LI) | DMSO-Nascent | 50 | 0.366029526 | 43.923543 | 439.2354315 | 439.2354315 | 21.961772 | 58.0 | 800 | 0.4 | 0.9 | 50.0 | VH306773 | 127N |
| SB47 | Input (LI) | DMSO-1h | 50 | 0.336595169 | 40.39142 | 403.914203 | 403.914203 | 20.19571 | 59.8 | 800 | 0.4 | 0.8 | 42.3 | VH306773 | 127C |
| SB47 | Input (LI) | DMSO-2h | 50 | 0.37223718 | 44.668462 | 446.6846155 | 446.6846155 | 22.334231 | 57.7 | 800 | 0.4 | 0.9 | 50.0 | VH306773 | 128N |
| SB47 | Input (LI) | 1uM TPL-Nascent | 50 | 0.314468362 | 37.736203 | 377.3620344 | 377.3620344 | 18.868102 | 61.1 | 800 | 0.4 | 0.8 | 50.0 | VH306773 | 128C |
| SB47 | Input (LI) | 1uM TPL-1h | 50 | 0.274120529 | 32.894464 | 328.9446351 | 328.9446351 | 16.447232 | 63.6 | 800 | 0.3 | 0.7 | 50.0 | VH306773 | 129N |
| SB47 | Input (LI) | 1uM TPL-2h | 50 | 0.266733868 | 32.008064 | 320.0806421 | 320.0806421 | 16.004032 | 64.0 | 800 | 0.3 | 0.6 | 50.0 | VH306773 | 129C |
| SB47 | Input (LI) | 50uM DRB-Nascent | 50 | 0.463523497 | 55.62282 | 556.2281967 | 556.2281967 | 27.81141 | 52.2 | 800 | 0.6 | 1.1 | 49.3 | VH306773 | 130N |
| SB47 | Input (LI) | 50uM DRB-1h | 50 | 0.406353115 | 48.762374 | 487.6237384 | 487.6237384 | 24.381187 | 55.6 | 800 | 0.5 | 1.0 | 36.0 | VH306773 | 130C |
| SB47 | Input (LI) | 50uM DRB-2h | 50 | 0.334701612 | 40.164193 | 401.6419339 | 401.6419339 | 20.082097 | 59.9 | 800 | 0.4 | 0.8 | 47.0 | VH306773 | 131 |
| SB45 | IP | Negative Control (no EdU) | 70 | 0.068579789 | 8.2295747 | 82.29574715 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | UL298812 | 126 |
| SB45 | IP | DMSO-Nascent | 70 | 0.070182423 | 8.4218908 | 84.2189075 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | UL298812 | 127N |
| SB45 | IP | DMSO-1h | 70 | 0.089075891 | 10.689107 | 106.8910694 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | UL298812 | 127C |
| SB45 | IP | DMSO-2h | 70 | 0.065319655 | 7.8383586 | 78.38358577 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | UL298812 | 128N |
| SB45 | IP | 1uM TPL-Nascent | 70 | 0.102055182 | 12.246622 | 122.4662184 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | UL298812 | 128C |
| SB45 | IP | 1uM TPL-1h | 70 | 0.074191559 | 8.9029871 | 89.02987074 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | UL298812 | 129N |
| SB45 | IP | 1uM TPL-2h | 70 | 0.061817084 | 7.41805 | 74.18050044 | 200 | 10 | 50 | 800 | 0.1 | 0.1 | 50 | UL298812 | 129C |
| SB45 | IP | 50uM DRB-Nascent | 70 | 0.07267314 | 8.7207768 | 87.20776818 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | UL298812 | 130N |
| SB45 | IP | 50uM DRB-1h | 70 | 0.055164113 | 6.6196935 | 66.19693509 | 200 | 10 | 50 | 800 | 0.1 | 0.1 | 50 | UL298812 | 130C |
| SB45 | IP | 50uM DRB-2h | 70 | 0.042016392 | 5.0419671 | 50.41967054 | 200 | 10 | 50 | 800 | 0.1 | 0.1 | 50 | UL298812 | 131 |
| SB46 | IP | Negative Control (no EdU) | 70 | 0.095474942 | 11.456993 | 114.5699302 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | VH306773 | 126 |
| SB46 | IP | DMSO-Nascent | 70 | 0.115233526 | 13.828023 | 138.2802312 | 200 | 10 | 50 | 800 | 0.1 | 0.3 | 50 | VH306773 | 127N |
| SB46 | IP | DMSO-1h | 70 | 0.118442621 | 14.213115 | 142.1311454 | 200 | 10 | 50 | 800 | 0.1 | 0.3 | 50 | VH306773 | 127C |
| SB46 | IP | DMSO-2h | 70 | 0.100628634 | 12.075436 | 120.7543607 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | VH306773 | 128N |
| SB46 | IP | 1uM TPL-Nascent | 70 | 0.103721615 | 12.446594 | 124.4659377 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | VH306773 | 128C |
| SB46 | IP | 1uM TPL-1h | 70 | 0.080112116 | 9.613454 | 96.13453956 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | VH306773 | 129N |
| SB46 | IP | 1uM TPL-2h | 70 | 0.077829384 | 9.3395261 | 93.395261 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | VH306773 | 129C |
| SB46 | IP | 50uM DRB-Nascent | 70 | 0.077981226 | 9.3577471 | 93.57747126 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | VH306773 | 130N |
| SB46 | IP | 50uM DRB-1h | 70 | 0.068607861 | 8.2329433 | 82.32943308 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | VH306773 | 130C |
| SB46 | IP | 50uM DRB-2h | 70 | 0.070472071 | 8.4566485 | 84.56648505 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | VH306773 | 131 |
| SB47 | IP | Negative Control (no EdU) | 70 | 0.116698354 | 14.003802 | 140.0380243 | 200 | 10 | 50 | 800 | 0.1 | 0.3 | 50 | VH306773 | 126 |
| SB47 | IP | DMSO-Nascent | 70 | 0.089106515 | 10.692782 | 106.9278177 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | VH306773 | 127N |
| SB47 | IP | DMSO-1h | 70 | 0.078348709 | 9.4018451 | 94.0184507 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | VH306773 | 127C |
| SB47 | IP | DMSO-2h | 70 | 0.068632105 | 8.2358525 | 82.35852547 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | VH306773 | 128N |
| SB47 | IP | 1uM TPL-Nascent | 70 | 0.12513132 | 15.015758 | 150.1575838 | 200 | 10 | 50 | 800 | 0.2 | 0.3 | 50 | VH306773 | 128C |
| SB47 | IP | 1uM TPL-1h | 70 | 0.11351733 | 13.62208 | 136.220796 | 200 | 10 | 50 | 800 | 0.1 | 0.3 | 50 | VH306773 | 129N |
| SB47 | IP | 1uM TPL-2h | 70 | 0.11853832 | 14.224598 | 142.2459838 | 200 | 10 | 50 | 800 | 0.1 | 0.3 | 50 | VH306773 | 129C |
| SB47 | IP | 50uM DRB-Nascent | 70 | 0.156547277 | 18.785673 | 187.8567327 | 200 | 10 | 50 | 800 | 0.2 | 0.4 | 50 | VH306773 | 130N |
| SB47 | IP | 50uM DRB-1h | 70 | 0.10459949 | 12.551939 | 125.5193885 | 200 | 10 | 50 | 800 | 0.1 | 0.3 | 50 | VH306773 | 130C |
| SB47 | IP | 50uM DRB-2h | 70 | 0.0933415 | 11.20098 | 112.0097995 | 200 | 10 | 50 | 800 | 0.1 | 0.2 | 50 | VH306773 | 131 |

TMT labelling:

* IPs (all of each batch) and Input (62 ug for SB45, 78.2 ug for SB46 and 61 ug for SB46) peptides were labelled
* Dry samples in speed vac
* Redissolve peptides in 50 µL 100 mM HEPES pH 8.5
* Take the same peptide amount (xx µL for IP and xx uL for Input) for all samples
* Add 100 mM HEPES pH 8.5 to reach 50 uL in total volume
* Add 45uL ACN to 800ug TMT tags, 400 ug TMT tags for Input (22 uL), 400 µg TMT tags for IP (22uL) with f.c. 30% of ACN
* Incubate samples (2 h, 22 ºC, 600 rpm)
* Quench unreacted TMT with 5 µL 5% hydroxylamine (30 min, 22 ºC, 600 rpm)
* Combine TMT-labelled samples in one tube (2 mL)
* Dry samples in speed vac (45 ºC)
* High pH reversed phase fractionation:
  + Re-suspend TMT-labelled peptides in 220 µL 5% formic acid, centrifuge and transfer into vials
  + Column: Waters XBridge Peptide BEH C18, 130Å, 3.5 µm, 4.6 mm × 250 mm
  + Solvents: A – 100% water, B – 100% ACN, C – 100 mM NH4 formate, pH 9.0
  + Gradient: (RP-hpH\_TMT\_48to24\_1MLMIN\_GA1\_tri\_long loading)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Retention [min] | Flow [ml/min] | %B | %C | %D |
| 1 | 0.000 | 1.000 | 5.0 | 10.0 | 0.0 |
| 2 | 8.000 | 1.000 | 5.0 | 10.0 | 0.0 |
| 3 | 10.000 | 1.000 | 21.5 | 10.0 | 0.0 |
| 4 | 21.000 | 1.000 | 48.8 | 10.0 | 0.0 |
| 5 | 22.000 | 1.000 | 90.0 | 10.0 | 0.0 |
| 6 | 27.000 | 1.000 | 90.0 | 10.0 | 0.0 |
| 7 | 28.000 | 1.000 | 5.0 | 10.0 | 0.0 |
| 8 | 35.000 | 1.000 | 5.0 | 10.0 | 0.0 |

* + 48 fractions (21 s each, from 9 to 26 min) into 24 fractions
  + Dry fractions *in vacuo*
  + Re-dissolve peptides in 30 µL 5% formic acid (15 µL load for IP, 3 uL load for Input)
* LC-MS/MS:
  + Thermo Orbitrap Fusion
  + 200 min run (PT10443)
  + SPS-MS3 scan method