A Targeted Proteomics Approach for Profiling Murine Cytochrome P450 Expression

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Supplemental Table 1. SRM assay transition list. The cytochrome P450s and transitions monitored for the targeted mass spectrometric assay are outlined below. The three highest intensity transitions per peptide monitored were included in the final assay. The three transitions were used for qualitative assessment of the limit of detection, based on signal-to-noise ratio of the three transitions and consistent presence in replicates. MRMs are represented as parent/fragment in the order of highest transition m/z to lowest. The asterisks denote enzymes for which heavy labeled (^{13}C and ^{15}N) synthetic peptides were included in the assay for quantitation.

Isozyme	MRM1 (transition)	MRM2 (transition)	MRM3 (transition)
1A2	743.390/1008.588 (y8)	743.390/894.545 (y7)	743.390/747.476 (y6)
*1A2	747.398/1016.602 (y8)	747.398/902.559 (y7)	747.398/755.491 (y6)
2A12	730.344/1047.449 (y9)	730.344/932.422 (y8)	730.344/795.363 (y7)
2B9	586.303/1056.572 (y8)	586.303/893.509 (y7)	586.303/780.425 (y6)
2B10	574.322/819.461 (y7)	574.322/621.324 (y5)	574.322/500.296 (y4)
*2B10	578.329/827.475 (y7)	578.329/629.338 (y5)	578.329/500.296 (y4)
2C29	636.815/1045.495 (y9)	636.815/830.404 (y7)	636.815/743.372 (y6)
2C37	473.820/783.57 (y7)	473.820/712.533 (y6)	473.820/599.449 (y5)
2C39	426.253/704.430 (y6)	426.253/603.382 (y5)	426.253/490.298 (y4)
2C50	673.886/975.583 (y9)	673.886/862.499 (y8)	673.886/761.452 (y8)
2C54	693.344/973.449 (y9)	693.344/858.422 (y8)	693.344/721.363 (y7)
2C67	482.285/616.378 (y5)	482.285/503.294 (y4)	482.285/247.144 (b2)
2C70	733.836/1153.501 (y10)	733.836/1040.417 (y9)	733.836/797.331 (y7)
2D9	629.825/828.473 (y6)	629.825/642.393 (y5)	629.825/385.256 (y3)
2D10	606.840/780.473 (y7)	606.840/709.436 (y6)	606.840/504.245 (b5)

2D22	599.796/695.383 (y6)	599.796/810.410 (y7)	599.796/369.224 (y3)
*2D22	604.800/705.392 (y6)	604.800/820.419 (y7)	604.800/379.233 (y3)
2D26	679.367/975.468 (y8)	679.367/904.431 (y7)	679.367/454.302 (b5)
2E1	623.833/963.468 (y8)	623.833/816.400 (y7)	623.833/702.357 (y6)
2F2	553.309/890.519 (y8)	553.309/775.492 (y7)	553.309/662.408 (y6)
2J5	763.406/1069.531 (y9)	763.406/1012.510 (y8)	763.406/898.467 (y7)
2U1	554.805/878.513 (y8)	554.805/731.445 (y7)	554.805/618.361 (y6)
3A11	692.851/1143.553 (y10)	692.851/786.399 (y7)	692.851/358.208 (y3)
*3A11	696.858/1151.567 (y10)	696.858/794.413 (y7)	696.858/366.223 (y3)
3A13	700.856/1073.526 (y9)	700.856/986.494 (y8)	700.856/839.426 (y7)
3A25	396.255/644.434 (y6)	396.255/573.397 (y5)	396.255/460.313 (y4)
4A10	621.323/1000.532 (y8)	621.323/887.448 (y7)	621.323/862.431 (b8)
4A12A	934.973/1207.595 (y10)	934.973/1078.553 (y9)	934.973/963.526 (y8)
4B1	463.289/755.466 (y7)	463.289/642.382 (y6)	463.289/543.314 (y5)
4F13	682.372/995.531 (y9)	682.372/848.462 (y8)	682.372/735.378 (y7)
4V2	566.829/886.515(y8)	566.829/702.430 (y6)	566.829/603.361 (y5)
*4V2	571.833/896.523 (y8)	571.833/712.438 (y6)	571.833/613.370 (y5)

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Supplemental Table 2. Lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) of the proteomics assay for peptides representing each cytochrome P450 protein. At least 6 peptide dilutions were found to be linear between the reported LLOQ and the ULOQ using a $1/y^2$ weighted regression for all peptides except CYPs 2C37 and 4A10 which were calibrated across 5 dilutions. Asterisks indicate a peptide with a heavy label (^{13}C and ^{15}N).

CYP	LLOQ (fmol)	ULOQ (fmol)
1A2*	3	600
2A12	3	30000
2B9	3	30000
2B10*	3	600
2C29	3	30000
2C37	30	3000
2C39	3	30000
2C50	60	30000
2C54	6	30000
2C67	30	30000
2C70	0.6	30000
2D9	6	30000
2D10	3	30000
2D22*	0.6	600
2D26	60	30000
2E1	60	30000
2F2	3	30000
2J5	15	30000
2U1	3	30000
3A11*	3	600
3A13	3	30000
3A25	15	30000
4A10	300	30000
4A12A	30	30000
4B1	3	30000
4F13	60	30000
4V2*	3	600