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| **In solution dimethyl labelling**  Modified according to Paul J Boersema et al. Multiplex peptide stable isotope labelling for quantitative proteomics. Nature Protocols 2009  Last modified 19.03.2014 |
| **Materials** |
| * TEAB (triethylammonium bicarbonate): 100mM ~pH 8.0 in water. 100 μl/sample * **\***Formaldehyde 4% (v/v) in water. 4 μl/sample   + CH2O (light labelling)   + CD2O (medium labelling)   + 13CD2O (heavy labelling) * **\***Sodium cyanoborohydride: 0.6M in water. 4 μl/sample   + NaBH3CN (light/intermediate labelling)   + NaBD3CN (heavy labelling) * **\***Ammonia: 1% (v/v) in water. 16 μl/sample * \*Trifluoroacetic acid: 100% (v/v) in water. 5 μl/sample   \*Prepare under fume hood |

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| **Procedure** | **Comments** |
| 1. Perform protein digestion (e.g. via FASP or in solution digestion). | * **Avoid the use of primary amine containing molecules upstream of dimethyl labelling** to maximize labelling efficiency (e.g. in steps involving ammonium bicarbonate, use TEAB instead) |
| 1. Determine the concentration of proteins in each sample. The in solution protocol is optimized for **up to 25 μg of protein** per label. |  |
| 1. Reconstitute the digested samples in 100 μl TEAB |  |
| 1. Add 4 μl of 4%    * **CH2O: light** labelling    * **CD2O: medium** labelling    * **13CD2O: heavy** labelling 2. Vortex and quickly spin down 3. Add 4 μl of 0.6M    * **NaBH3CN: light/medium** labelling    * **NaBD3CN: heavy** labelling 4. Incubate at room temperature for 1 h while shaking | * Perform all subsequent steps under fume hood * Initiation of labelling reaction |
| 1. Add 16 μl of 1% ammonia to stop the reaction 2. Vortex and quickly spin down 3. Mix the differentially labelled sample pairs 4. Add 5 μl of 100% TFA | * Formaldehyde/cyanoborohydride reacts with primary amines. The addition of ammonia, a primary amine, quenches the reaction |
| 1. Load and elute samples on stage tips and analyse by LC-MS |  |