**Supplementary Table 1.** Buffers for MTHFD2 and MTHFD1 DC-domain lysis, purification and inhibition assay.

|  |  |
| --- | --- |
| **Buffer** | **Buffer composition** |
| Lysis buffer A2 | BugBuster protein extraction reagent (Thermo Scientific) supplemented with complete protease inhibitor cocktail (Roche), Benzonase (Novagen, 2.5 U/mL) and lysozyme (Sigma, 0.5 mg/mL) |
| Buffer B2 | 50 mM Tris-HCl pH 7.8, 250 mM NaCl, 5 % glycerol |
| Buffer C2 | 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 10 % glycerol, 1 mM TCEP |
| Buffer D2 | 20 mM Tris-HCl pH 8.2, 20 mM NaCl, 15 % glycerol, 1 mM TCEP |
| Lysis buffer A1 | 50 mM Tris-HCl pH 7.8, 250 mM NaCl, 5 % glycerol, Protease Inhibitor Cocktail Set III (EDTA-Free, Calbiochem catalog no. 539134) 0.1 µL/mL buffer, benzonase nuclease 2.5 U/mL buffer and 0.5 mM TCEP |
| Buffer B1 | 50 mM Tris-HCl pH 7.8, 250 mM NaCl, 5 % glycerol, 10 mM imidazole and 0.5 mM TCEP |
| Buffer C1 | 50 mM Tris-HCl pH 7.8, 250 mM NaCl, 5 % glycerol, 500 mM imidazole and 0.5 mM TCEP |
| Buffer D1 | 50 mM Tris-HCl pH 7.8, 250 mM NaCl, 5 % glycerol and 0.5 mM TCEP |
| Buffer E1 | 20 mM Tris-HCl pH 8.3, 20 mM NaCl, 15% glycerol and 1 mM TCEP |
| MTHFD2 Assay buffer | 50 mM Tris pH 8.0, 100 mM NaCl, 25 mM NaPi, 5 mM MgCl2, 20 mM ß-mercaptoethanol, 0.005 % Tween 20 and 250 µM NAD+ |
| MTHFD1 Assay buffer | 25 mM MOPS pH 7.3, 0.5 mM TCEP and 400 µM NADP+ |
| DSF Buffer | 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 25 mM NaPi, 5 mM MgCl2 and 1 mM TCEP. |
| TBS | 50 mM Tris-C pH 7.5 and 150 mM NaCl |
| 10x TN buffer | 500 mM Tris-HCl, pH 8.0, 500 mM NaCl |