# Differential scanning fluorimetry – Melting temperatures and fitting statistics

Table 1. Summary of the DSF results obtained with DocSTm at 5 µM. In all cases the sigmoidal fit was applied in the temperature range of 30.0 to 52.0 °C.

|  |  |  |
| --- | --- | --- |
| **Replicate** | **Tm (°C)** | **R2** |
| 1 | 42.46 | 0.9997 |
| 2 | 41.91 | 0.9997 |
| 3 | 41.48 | 0.9993 |
| 4 | 44.47 | 0.9996 |
| 5 | 43.57 | 0.9996 |
| 6 | 42.59 | 0.9995 |
| 7 | 42.89 | 0.9996 |
| 8 | 43.53 | 0.9993 |
| 9 | 43.06 | 0.9997 |
| **Average** | **42.9 ± 0.9** | **-** |

Table 2. Summary of the DSF results of DocSTm at 5 µM in the presence of 50 µM of PhdSTm1-73 and peptides 1–23.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Binder** | **Fitting range (°C)** |  | **Replicate 1** | |  | **Replicate 2** | |  | **Replicate 3** | |  | **Average Tm (°C)** |
|  | **Tm (°C)** | **R2** |  | **Tm (°C)** | **R2** |  | **Tm (°C)** | **R2** |  |
| Phd1-73 | 65.7 - 78.4 |  | 73.6 | 0.9993 |  | 73.9 | 0.9993 |  | 74.9 | 0.9994 |  | 74.2 ± 0.7 |
| **1** (PhdSTm52-73) | 63.2 - 76.0 |  | 71.8 | 0.9996 |  | 71.5 | 0.9996 |  | 71.6 | 0.9997 |  | 71.7 ± 0.1 |
| **2** (PhdSTm52-73-M52A) | 63.2 – 74.7 |  | 71.3 | 0.9995 |  | 70.8 | 0.9995 |  | 70.6 | 0.9995 |  | 70.9 ± 0.4 |
| **3** (PhdSTm52-73-D53A) | 59.1 – 72.9 |  | 68.3 | 0.9997 |  | 68.4 | 0.9997 |  | 68.8 | 0.9997 |  | 68.5 ± 0.3 |
| **4** (PhdSTm52-73-D54A) | 61.5 – 72.5 |  | 69.0 | 0.9994 |  | 69.3 | 0.9993 |  | 69.1 | 0.9994 |  | 69.1 ± 0.2 |
| **5** (PhdSTm52-73-E55A) | 59.6 – 72.9 |  | 66.3 | 0.9997 |  | 66.3 | 0.9997 |  | 66.2 | 0.9996 |  | 66.3 ± 0.1 |
| **6** (PhdSTm52-73-F56A) | 46.7 – 70.4 |  | 64.5 | 0.9957 |  | 64.1 | 0.9963 |  | 63.5 | 0.9968 |  | 64.0 ± 0.5 |
| **7** (PhdSTm52-73-I59A) | 48.0 – 67.7 |  | 59.7 | 0.9986 |  | 57.7 | 0.9987 |  | 57.6 | 0.9993 |  | 58.4 ± 1.2 |
| **8** (PhdSTm52-73-M60A) | 54.6 – 68.5 |  | 64.1 | 0.9994 |  | 63.3 | 0.9994 |  | 63.7 | 0.9995 |  | 63.7 ± 0.4 |
| **9** (PhdSTm52-73-V62A) | 58.0 – 72.9 |  | 68.3 | 0.9997 |  | 67.4 | 0.9997 |  | 66.9 | 0.9996 |  | 67.5 ± 0.7 |
| **10** (PhdSTm52-73-H63A) | 51.1 – 68.9 |  | 63.3 | 0.9991 |  | 63.7 | 0.9996 |  | 64.2 | 0.9995 |  | 63.7 ± 0.4 |
| **11** (PhdSTm52-73-G64A) | 59.0 – 74.7 |  | 68.8 | 0.9995 |  | 69.2 | 0.9997 |  | 68.9 | 0.9997 |  | 69.0 ± 0.2 |
| **12** (PhdSTm52-73-N65A) | 60.7 – 75.2 |  | 71.8 | 0.9996 |  | 71.0 | 0.9997 |  | 70.4 | 0.9993 |  | 71.1 ± 0.7 |
| **13** (PhdSTm52-73-E66A) | 62.0 – 74.5 |  | 70.8 | 0.9996 |  | 70.4 | 0.9995 |  | 70.6 | 0.9995 |  | 70.6 ± 0.2 |
| **14** (PhdSTm52-73-L67A) | 47.2 – 64.5 |  | 57.4 | 0.9998 |  | 57.2 | 0.9998 |  | 57.0 | 0.9998 |  | 57.2 ± 0.2 |
| **15** (PhdSTm52-73-R68A) | 62.2 – 74.5 |  | 70.6 | 0.9997 |  | 70.7 | 0.9996 |  | 70.4 | 0.9997 |  | 70.6 ± 0.1 |
| **16** (PhdSTm52-73-E69A) | 63.6 – 78.2 |  | 73.5 | 0.9996 |  | 73.5 | 0.9996 |  | 73.9 | 0.9996 |  | 73.6 ± 0.2 |
| **17** (PhdSTm52-73-L70A) | 47.5 – 65.5 |  | 58.5 | 0.9997 |  | 57.9 | 0.9997 |  | 57.8 | 0.9997 |  | 58.0 ± 0.4 |
| **18** (PhdSTm52-73-D72A) | 60.0 – 74.9 |  | 71.1 | 0.9996 |  | 71.0 | 0.9996 |  | 70.6 | 0.9992 |  | 70.9 ± 0.3 |
| **19** (PhdSTm52-73-K73A) | 57.0 – 72.7 |  | 68.8 | 0.9997 |  | 67.6 | 0.9997 |  | 67.7 | 0.9997 |  | 68.0 ± 0.6 |
| **20** (PhdSTm52-71) | 58.0 – 72.5 |  | 68.5 | 0.9995 |  | 68.4 | 0.9994 |  | 68.5 | 0.9994 |  | 68.5 ± 0.1 |
| **21** (PhdSTm55-73) | 53.3 – 66.5 |  | 62.3 | 0.9995 |  | 62.0 | 0.9995 |  | 62.4 | 0.9995 |  | 62.2 ± 0.2 |
| **22** (PhdSTm55-71) | 43.5 – 55.7 |  | 58.89 | 0.9995 |  | 59.16 | 0.9995 |  | 59.42 | 0.9994 |  | 59.2 ± 0.3 |
| **23** (PhdSTm56-70) | 52.0 – 63.7 |  | 50.54 | 0.9995 |  | 50.60 | 0.9996 |  | 51.08 | 0.9996 |  | 50.7 ± 0.3 |

Table 3. Summary of the DSF results of DocSTm at 5 µM in the presence of multiple combinations of peptides 24 (PhdSTm52-63) and 25 (PhdSTm65-73).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Peptide 24** | **Peptide 25** | **Fitting range (°C)** |  | **Replicate 1** | |  | **Replicate 2** | |  | **Replicate 3** | |  | **Average Tm (°C)** |
|  | **Tm (°C)** | **R2** |  | **Tm (°C)** | **R2** |  | **Tm (°C)** | **R2** |  |
| 50 µM | - | 39.1 – 51.7 |  | 46.1 | 0.9989 |  | 46.1 | 0.9989 |  | 45.8 | 0.9982 |  | 46.0 ± 0.1 |
| - | 50 µM | 37.1 – 50.7 |  | 44.1 | 0.9986 |  | 44.2 | 0.9986 |  | 44.4 | 0.9973 |  | 44.2 ± 0.1 |
| 100 µM | - | 38.1 – 50.2 |  | 46.1 | 0.9993 |  | 46.4 | 0.9991 |  | 46.6 | 0.9981 |  | 46.3 ± 0.3 |
| - | 100 µM | 35.5 - 50.1 |  | 43.2 | 0.9992 |  | 43.2 | 0.9995 |  | 43.4 | 0.9993 |  | 43.3 ± 0.1 |
| 100 µM | 25 µM | 40.0 – 52.1 |  | 46.1 | 0.9992 |  | 46.1 | 0.9991 |  | 46.2 | 0.9984 |  | 46.2 ± 0.1 |
| 100 µM | 50 µM | 39.0 – 52.0 |  | 46.2 | 0.9993 |  | 46.2 | 0.9992 |  | 46.6 | 0.9969 |  | 46.3 ± 0.2 |
| 100 µM | 100 µM | 39.7 – 53.1 |  | 46.0 | 0.9994 |  | 46.3 | 0.9993 |  | 46.8 | 0.9984 |  | 46.4 ± 0.4 |
| 50 µM | 100 µM | 39.1 – 52.0 |  | 45.5 | 0.9991 |  | 45.7 | 0.9990 |  | 46.0 | 0.9974 |  | 45.7 ± 0.2 |
| 25 µM | 100 µM | 39.6 – 50.5 |  | 44.9 | 0.9987 |  | 45.1 | 0.9990 |  | 45.2 | 0.9986 |  | 45.1 ± 0.1 |