Choice of Buffers For a DSC Scan

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Although it is often assumed that the pH of a buffer solution remains constant during a differential scanning calorimetry (DSC) experiment, the pK of a buffer can in fact change significantly between 0 °C and 100 °C. Such pH changes can result in sample denaturation, aggregation and precipitation that, in the absence of knowledge about the temperature stability of the buffer, could erroneously be ascribed solely to temperature-induced unfolding of the biomolecule.

The table below is a compilation of calculated pK values of common biological buffers as a function of temperature:

Phosphate produces the most temperature-stable buffer in the physiological pH range 7.0 to 7.7. All buffers should be prepared using thoroughly-degassed ultra-pure water, and be briefly degassed again immediately prior to use. Addition of thermally unstable additives such as bacteriocides should be avoided. Dithiothreitol is preferable as a reducing agent to mercaptoethanol, which oxidizes and thermally denatures easily.

Temp (°C)	acetate	MES	PIPES	Phos	BES	MOPS	TES	HEPES	Tris	Bicine	TAPS	CAPS
0	4.73	6.35	6.98	6.92	7.40	7.44	7.93	7.73	8.98	8.63	8.97	11.06
10	4.71	6.24	6.90	6.86	7.23	7.30	7.71	7.58	8.65	8.45	8.69	10.74
20	4.70	6.15	6.84	6.82	7.08	7.16	7.50	7.46	8.34	8.28	8.42	10.43
30	4.70	6.06	6.77	6.79	6.93	7.04	7.31	7.34	8.06	8.12	8.18	10.15
40	4.70	5.97	6.70	6.77	6.79	6.91	7.13	7.22	7.81	7.97	7.95	9.88
50	4.71	5.89	6.64	6.76	6.86	6.80	6.98	7.11	7.57	7.83	7.74	9.63
60	4.73	5.81	6.58	6.78	6.54	6.69	6.81	7.00	7.35	7.70	7.54	9.39
70	4.75	5.74	6.53	6.77	6.42	6.58	6.66	6.89	7.15	7.58	7.38	9.16
80	4.78	5.67	6.47	6.79	6.31	6.48	6.53	6.79	6.96	7.46	7.18	8.94
90	4.81	5.60	6.42	6.82	6.21	6.38	6.40	6.69	6.76	7.35	7.02	8.74
100	4.84	5.53	6.37	6.85	6.11	6.28	6.29	6.60	6.62	7.25	6.87	8.84

(Phos: phosphate. Values from H. Fukada and K. Takahashi (1987), Laboratory of Biophysical Chemistry, College of Agriculture, Sakai, Osaka, Japan.)