

**Table 3. Determination of Cdc5-dependent phosphorylation sites onto GST-Swe1(K473A) *in vitro***

Peptide	Sequence	Amino acids
1	QAGEDES*DDFAIGGSTPTNK	30–49
2	IEEEEEEEEEEGKDEES*VDSR	87–106
3	RWS*PFHENESVTTTPITK	109–125
4	WSPFHENES*VTTTPITK	110–125
5	SAEKT*NSPISLK	127–138
6	TENTS*SSSSYSVAK	152–165
7	PNQS*AFTSSGLVSK	166–179
8	<u>M</u> <u>S</u> <u>M</u> <u>D</u> <u>T</u> <u>S</u> <u>L</u> <u>Y</u> <u>P</u> <u>A</u> <u>K</u> 1P	180–190
9	<u>N</u> <u>A</u> <u>S</u> <u>S</u> <u>S</u> <u>L</u> <u>S</u> <u>V</u> <u>S</u> <u>P</u> <u>L</u> <u>N</u> <u>F</u> <u>V</u> <u>E</u> <u>D</u> <u>N</u> <u>N</u> <u>L</u> <u>Q</u> <u>E</u> <u>D</u> <u>L</u> <u>L</u> <u>F</u> <u>S</u> <u>D</u> <u>S</u> <u>P</u> <u>S</u> <u>S</u> <u>K</u> 2P (S)	219–250
10	<u>N</u> <u>A</u> <u>S</u> <u>S</u> <u>S</u> <u>L</u> <u>S</u> <u>*V</u> <u>S</u> <u>P</u> <u>L</u> <u>N</u> <u>F</u> <u>V</u> <u>E</u> <u>D</u> <u>N</u> <u>N</u> <u>L</u> <u>Q</u> <u>E</u> <u>D</u> <u>L</u> <u>L</u> <u>F</u> <u>S</u> <u>D</u> <u>S</u> <u>P</u> <u>S</u> <u>S</u> <u>K</u>	219–250
11	ALPS*IHVPTIDSSPLSEAK	251–269
12	HNNQT*NILSPTNSLVTNSSPQTLHSNK	276–302
13	HNNQTNILSP <u>T</u> <u>N</u> <u>S</u> <u>L</u> <u>V</u> <u>T</u> <u>N</u> <u>S</u> <u>S</u> <u>P</u> <u>Q</u> <u>T</u> <u>L</u> <u>H</u> <u>S</u> <u>N</u> <u>K</u> 1P (T or S)	276–302
14	RKS*IIGATSQTHR	377–389
15	ESRPLS*LSSAIVTNTTSAETHSISSTDSSPLNSK	390–423
16	LSANPDS*HLFEK	432–443
17	ILNEV <u>T</u> <u>N</u> <u>Q</u> <u>I</u> <u>T</u> <u>M</u> <u>D</u> <u>Q</u> <u>E</u> <u>G</u> <u>K</u> 1P (T)	492–507
18	LGDFGMATHLPLEDKS*FENEGDR	595–617
19	EYIAPEIISDCT*YDYK	618–633
20	LSSTDIHSESLFSDIT*KVDTNDLDFDFER	673–700
21	LSSTDIH <u>S</u> <u>E</u> <u>S</u> <u>L</u> <u>F</u> <u>S</u> <u>D</u> <u>I</u> <u>T</u> <u>*K</u> <u>V</u> <u>D</u> <u>T</u> <u>N</u> <u>D</u> <u>L</u> <u>F</u> <u>D</u> <u>F</u> <u>E</u> <u>R</u> 1P (S)	673–700

Phosphorylation sites were determined by nanoelectrospray tandem mass spectrometry. Definitive phosphosites are indicated by an asterisk. Underlined and italicized Ser or Thr indicates potential phosphosites. 1P or 2P indicates the number of phosphate groups in the given peptide, whereas S or T in parenthesis indicates the phosphorylated residues. A Swe1 derivative bearing mutations in the Cdc5 phosphorylation sites [Swe1(20A)] was generated by mutagenizing S36, S102, S111, S118, T131, S156, S169, S185, S223, S225, S254, T280, S288, S379, S395, S438, T501, S610, T629, S682, and T688 to Alanine (A). D. J. Lew and his colleagues (Duke University, Durham, NC) have determined three *in vivo* Swe1 phosphorylation sites, and these sites are identical to the phosphosites on peptides 3–5 (D. J. Lew, personal communication).