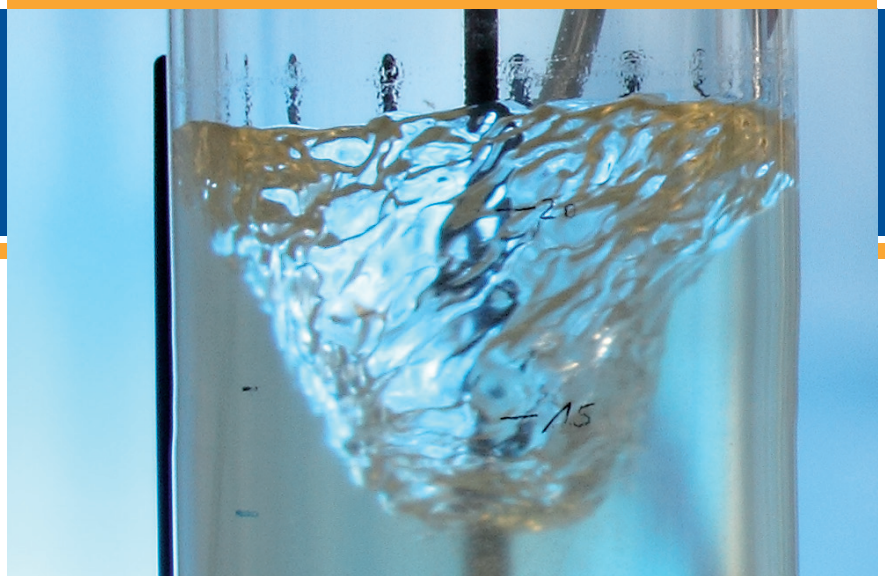
SYNTHETIC PEPTIDE SERVICES

Custom Synthesis of Difficult and Challenging Peptides

***emp** BIOTECH* offers a full and comprehensive range of synthetic peptide services, specialising in linear and circular peptides containing unusual building blocks and/or modifications.



Custom manufacture and design of high quality, high purity peptides

Special expertise in

- Fluorescence labeling
- Protease and kinase substrates
- Histone peptides
- Beta-amyloid peptides
- Modified ubiquitins

Automated, multiple solid-phase peptide synthesis

- Chain length up to 100 residues
- Scale from 1 to 500 mg
- Preparative HPLC purification (standard purity: 80% or 95%)
- Characterization of all products by HPLC and mass spectrometry

engineered molecular precision

Custom Synthesis of Difficult and Challenging Peptides

Special Substrates

- Symmetrical and Asymmetrical Rhodamine 110 Protease Substrates
- Fluorescently Labeled Ubiquitin and Nedd8
- Multiple phosphorylated kinase substrates[1]
- MALT1 substrates[2]
- MUC1 peptide[3]
- In vivo cleavable cell-permeable peptides[4]

Modified Peptides

- Residues with modified aliphatic and aromatic side chains, e.g. methyl-, dimethyl- and trimethyl-lysine, methyl- and dimethyl-arginine (sym. or asym.), hydroxyproline, citrulline, selenocysteine, -methionine, among others
- D-amino acids
- Phosphorylation of Tyr, Ser, Thr
- Glycosylated peptides with Asn(GlcNAc), Ser/Thr(GalNAc, TF, etc.)
- Chelator moieties (i.e. DOTA, NODA, NOTA, Hynic, etc.)
- Stable isotopes (e.g. ^{2}H, ^{13}C, ^{15}N)
- Depsipeptides
- Cyclic peptides (cyclized via backbone or via side chain)
- Peptides having multiple disulfide bridges
- Alkyne- / azide-modified peptides for click-chemistry
- Farnesylated Cys-peptides (e.g. -Cys(Farn)-OMe)
- Fatty acids / carboxylic acids (C1-C18, malonic acid, succinic acid and others)
- Peptide thioesters for chemical ligation
- Spin labels (TOAC, TEMPYO)

N- and C-terminal derivatization

- Biotinylation
- Hydrophobic (Ahx) or hydrophilic (PEG) spacers
- Acetyl-, formyl- and palmitoyl-peptides
- Peptide hydrazides, hydroxamates and alkylated peptide amides
- Selective linkers for peptide conjugates (maleimide, chloro- or bromo-acetyl)
- Cys(Pys/Npys) for synthesis of peptide conjugates or asymmetric cystine-peptides

Fluorescence labeling

- Fluorescein, rhodamine, cyanine and coumarin derivatives (e.g. FITC / FAM / Fluo, TAMRA, Cy5, AMC, AMCA, AFC and others)
- Labeling of peptides with single isomer DYOMICS-label (spectral range 400-800 nm), for more details see www.dyomics.com
- FRET-systems (Abz / Tyr(NO_2), Dnp / AMC, Dabcyl / Edans and others)
- Protease substrates (caspases, beta-secretase and others):
  - with AMC or AFC
  - with rhodamine 110 (Rh110)
  - symmetric: $(peptide)_2$-Rh110 or
  - asymmetric: peptide-Rh110-X (X = D-Pro or gamma-Glu)

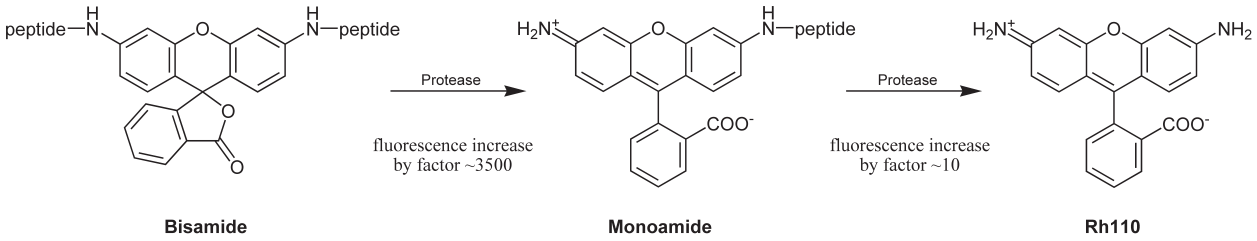
[1] N. Czudnochowski et al., Nature Commun., 2012, 3, 842.
[2] C. Wiesmann et al., J. Mol. Biol., 2012, 419, 4.
[3] U. Karsten et al., Glycobiol., 2004, 14, 681.
[4] H. Herce et al., Nature Commun., 2013, 4, 2660.

Symmetrical and Asymmetrical Rhodamine 110 Protease Substrates

Why Rh110-Protease Substrates?

Biological samples have an intrinsically high autofluorescence at blue and green wavelengths which can interfere with fluorescence measurements. This is particularly significant with fluorophores such as AMC, which absorb and emit in this region. Better suited for this purpose are red-shifted rhodamines, such as Rh110, which emit in a region of low autofluorescence to sufficiently reduce background noise and increase sensitivity. Rh110-based assays have been shown to deliver fewer false positives as compared to AMC labeled substrates[5] or proteins such as ubiquitin[6].

Furthermore, bis-substituted Rh110-substrates have an additional advantage: they are virtually non-fluorescent until after cleavage of an amide linkage to yield a highly fluorescent monoamide. Cleavage of the second amide yields free Rh110, which further increases fluorescence intensity[7].



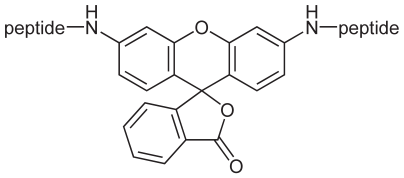
Bisamide **Monoamide** **Rh110**

Advantages:

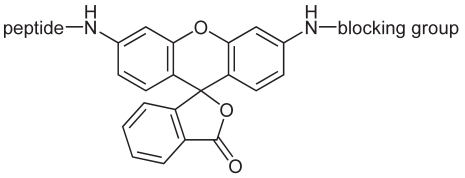
- Up to 300-fold higher sensitivity than analogous coumarin-derivatives
- Fewer false positives
- Rh110-substrates available for a wide range of proteases
- Uncleaved substrate has near zero fluorescence intensity
- Red-shifted excitation and emission wavelengths in the region of low sample autofluorescence:
  AMC: Ex 380 nm / Em 460 nm
  vs. Rh110: Ex 492 nm / Em 529 nm

Target	asymmetrical Rh110-substrate
caspase-3	Z-DEVD-Rh110-(D-Pro)
MALT1	Ac-LRSR-Rh110-(D-Pro)[8]
calpain	Succ-LLVY-Rh110-(D-Pro)
trypsin prostasin matriptase	Bz-QAR-Rh110-(D-Pro)

Two variations of Rh110-labeled substrates are available: **symmetrical and asymmetrical**



Symmetrical Rh110 substrates are used in order to achieve the maximum increase in fluorescence upon cleavage (e.g. for fluorescence microscopy or FACS).



For enzyme kinetic studies, the **asymmetrical Rh110 substrate** allows only one proteolytic cleavage, as the second amino group of Rh110 is blocked (i.e. by D-proline). This is essential for achieving high linear dynamic ranges and determining precise kinetic enzyme parameters.

[5] S.K. Grant, J.G. Sklar, R.T. Cummings, J. Biomol. Screen., 2002, 7(6), 531-540.
[6] U. Hassiepen et al., Anal.Biochem., 2007, 371, 201-207.
[7] S.P. Leytus, L.L. Melhado, W.F. Mangel, Biochem. J., 1983, 209, 299-307.
[8] C. Wiesmann et al., J. Mol. Biol., 2012, 419, 4.

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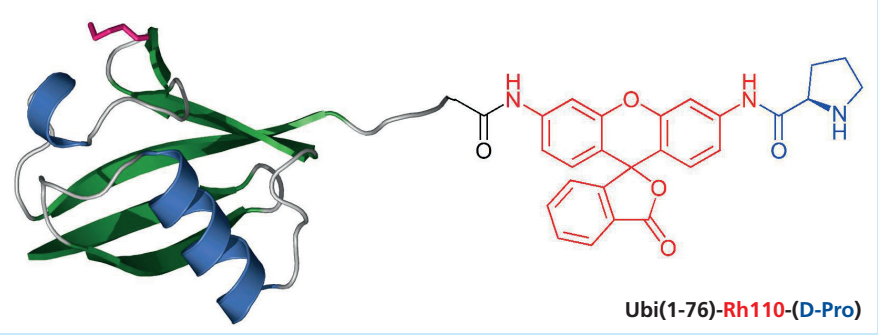
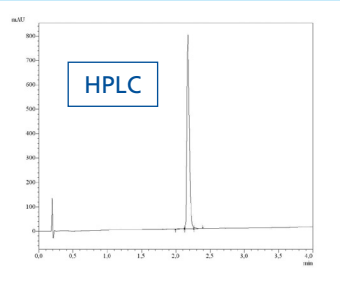
emp BIOTECH

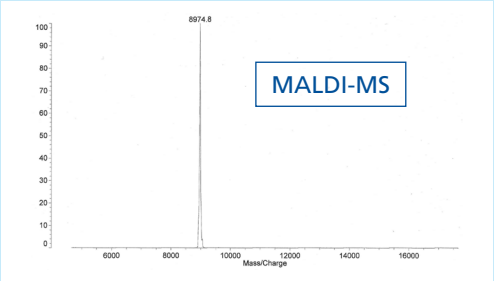
Fluorescently Labeled Ubiquitin



Fully synthetic bulk quantities, priced competitively

Purity:	95% (HPLC)
Sequence:	MQIFVKTLTG[10] KTITLEVEPS[20] DTIENVKAKI[30] QDKEGIPPDQ[40] QRLIFAGKQL[50] EDGRTLSDYN[60] IQKESTLHLV[70] LRLRGG[76]-Rh110-(D-Pro)
MW:	8974.37
Analytical data:	HPLC-Trace and MALDI-MS of purified Ubi(1-76)-Rh110-(D-Pro)





Other available fluorescent ubiquitin derivatives:

Ubiquitin(1-76)-Rh110-(D-Pro)	1 mg
Ubiquitin(1-76)-Rh110-Gly[9]	1 mg
Ubiquitin(1-76)-AMC	1 mg
Nedd8 derivatives available on request	

For more information, please contact us at **+49 30 9489 2201** or **tech@empbiotech.com**

[9] U. Hassiepen et al., Anal. Biochem., 2007, 371, 201-207.

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