

AGE-CML ASSAY

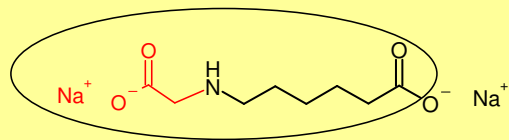
The antibody used in the assay is the mouse monoclonal 4G9

- Generated by immunization with KLH after browning with glucose
- Screened against BSA-AGE
- Epitope characterized to be 6-(N-carboxymethylamino)caproate
- Recognizes isolated epitope as well as N- ϵ -carboxymethyl-lysine in context of protein
- No cross-reactivity with non-derivatized lysine, glycine, alanine or β -alanine; very low cross-reactivity with sarcosin (0,06%)

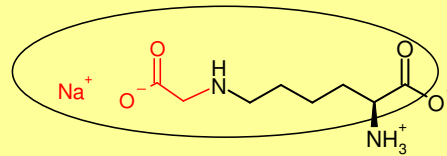


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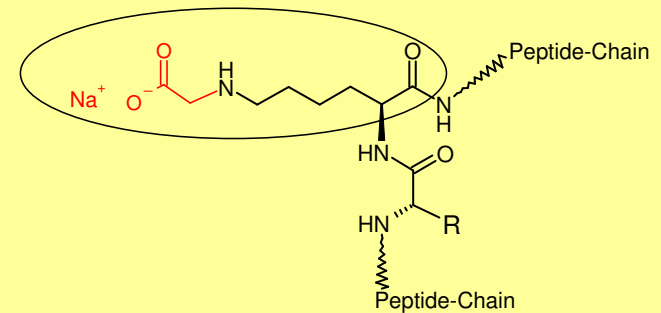
Epitope of the Anti-CML-MAb 4G9



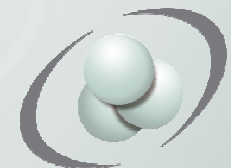
**N-Carboxymethyl
aminocaproic acid,
used as standard
in the assay**



**N-epsilon-
Carboxymethyl
Lysine (CML)**



**Carboxymethylated
(AGE) Protein**



AGE-CML ASSAY

Competitive Immunoassay Format

- streptavidin microplates, coated with biotinylated BSA-AGE
- simultaneous incubation of sample and MAbs anti-CML-HRP
- substrate reaction with ABTS / perborate
- calibration standard curve generated with increasing concentrations of 6-(N-carboxymethylamino)caproate (single epitope)

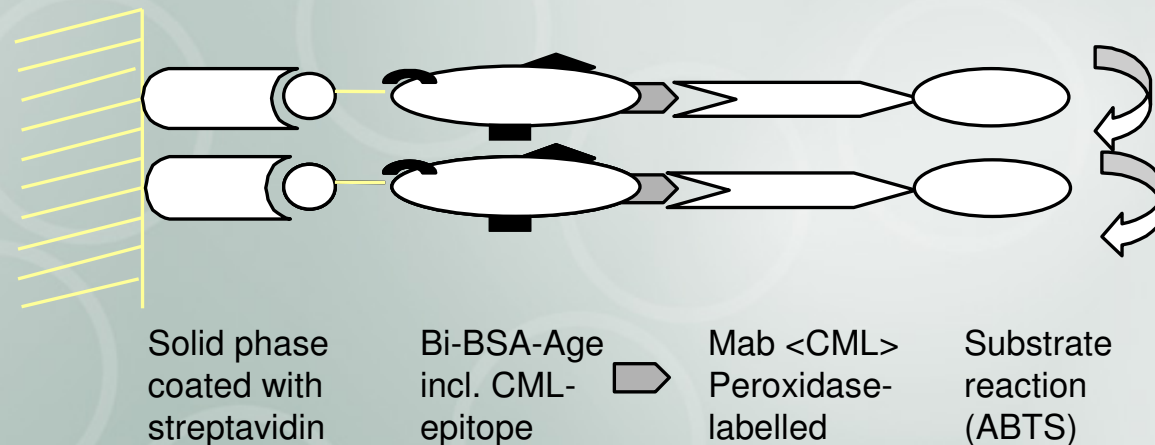
- results expressed as number of single epitopes in a sample, independent of the context of the epitopes
- sample material suitable for the assay: serum, urine, CSF, synovial fluid, tissue homogenates
- after proteolytic treatment (improved exposure of the epitope) samples reveal more detectable CML



AGE-CML ASSAY

Assay: Competitive ELISA on Microplates

Principle:



Competition with analyte from the sample, containing CML-Epitopes

Sample material: Serum, Plasma, Erythrocyte Lysate, others are evaluated

Higher signals are obtained after limited proteolysis of the samples with Proteinase K

