

Advantages of CCMS in comparison to other methods

Capture Compound	Activity based probes (ABP)	Affinity chromatography (Pull down)	Immune precipitation (Pull down)
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Overview

<p>- Trifunctional molecule</p> <p>• Capture Compounds consist of:</p> <ul style="list-style-type: none"> • Selectivity function • Reactivity function • Sorting function 	<p>- Bifunctional molecule</p> <p>• Bifunctional molecules consist of:</p> <ul style="list-style-type: none"> • Combined selectivity and reactivity function • Selectivity function • Sorting function 	<p>- Immobilized small molecules</p> <p>• Immobilized small molecules consist of:</p> <ul style="list-style-type: none"> • Selectivity function on solid support 	<p>- Antibody</p> <p>• Antibodies consist of:</p> <ul style="list-style-type: none"> • IgG fragments for specific protein targeting • Sorting function for isolation or detection
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Preparation

<p>• Advantages:</p> <ul style="list-style-type: none"> • Affinity based interaction between native protein and selectivity function • Equilibrium between target and selectivity function allows K_D-determination • Binding of low affinity-based, lipophilic and large proteins • In-solution, no limitation by solid phases • Competition 	<p>• Advantages:</p> <ul style="list-style-type: none"> • Affinity based interaction • Covalent cross-link <p>• Disadvantages:</p> <ul style="list-style-type: none"> • Immediate cross-link between selectivity function and active site of protein • Limited to active enzymes • No equilibrium between target and selectivity function • Increased false-positive binding • No competition possible 	<p>• Advantages:</p> <ul style="list-style-type: none"> • Affinity based interaction • Scalable to preparative range • Stable and automatable for high throughput <p>• Disadvantages:</p> <ul style="list-style-type: none"> • Interaction only in aqueous solution • No cross-link • Wash-out of low affinity bound proteins • Charge and salt dependent • Pore size dependency for protein-ligand interaction 	<p>• Advantages:</p> <ul style="list-style-type: none"> • In-solution • Competition <p>• Disadvantages:</p> <ul style="list-style-type: none"> • Recognition of specific amino acid sequence • Non-covalent affinity interaction • Low affinity bound proteins lost during washing steps • Cross-reactions of AB • Less stable • Limited to known targets • Limited to water soluble proteins • Extensive to prepare
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Cross-linking

<p>• Photo reactivity group is activated via UV light and resulting in irreversible immobilization of proteins to Capture Compound</p> <p>• Cross-link occurs outside the ligand binding site</p> <p>• Higher yield of low affinity bound proteins</p> <p>• Identification of the cross-linked amino acid via MS</p>	<p>- No comparable function</p>	<p>- No comparable function</p>	<p>- No comparable function</p>
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Isolation

<p>• Advantage:</p> <ul style="list-style-type: none"> • Reduction of biological complexity in-solution • Isolation of lipophilic proteins • Pull out via Streptavidin-biotin • Higher yields • Direct in-solution tryptic digestion for LC-MS analysis • Isolation of weak interacting partners • Automatable for high-throughput <p>• Disadvantage:</p> <ul style="list-style-type: none"> • Presence of biotinylated proteins 	<p>• Advantage:</p> <ul style="list-style-type: none"> • Reduction of biological complexity <p>• Disadvantage:</p> <ul style="list-style-type: none"> • Isolation efficiency depends on interaction on a solid phase 	<p>• Advantage:</p> <ul style="list-style-type: none"> • Reduction of biological complexity • Automatable for high-throughput <p>• Disadvantage:</p> <ul style="list-style-type: none"> • Isolation efficiency depends on elution efficiency • Less compatible with lipophilic proteins • Eluted proteins are diluted 	<p>• Advantage:</p> <ul style="list-style-type: none"> • Reduction of biological complexity in solution • Pull out via sepharose beads <p>• Disadvantage:</p> <ul style="list-style-type: none"> • IgG fragments disturb protein analysis • Weak interaction between beads and antibody • Loss of material during isolation steps • Separation of isolated proteins and antibody prior LC-MS analysis
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Ordering information

Capture Compounds are available as caproKits™ from caprotec bioanalytics. Currently available caproKits provide components with staurosporine, cAMP, and SAH selectivity functions to target kinases, methyltransferases and other SAM binding proteins.

Item Nr.	Description
1-1020-050	Stauro caproKit™ 50 reactions
1-1020-010	Stauro caproKit™ 10 reactions
1-1030-050	C8-cAMP caproKit™ 50 reactions
1-1030-010	C8-cAMP caproKit™ 10 reactions
1-1031-050	C2-cAMP caproKit™ 50 reactions
1-1031-010	C2-cAMP caproKit™ 10 reactions
1-1032-050	N ⁶ -cAMP caproKit™ 50 reactions
1-1032-010	N ⁶ -cAMP caproKit™ 10 reactions
1-1035-000	cAMP caproKit™ complete
1-1040-050	N ² -cGMP caproKit™ 50 reactions
1-1040-010	N ² -cGMP caproKit™ 10 reactions
1-1050-050	GDP caproKit™ 50 reactions
1-1050-010	GDP caproKit™ 10 reactions
1-1070-050	SAHA caproKit™ 50 reactions
1-1070-010	SAHA caproKit™ 10 reactions
1-1080-050	Dasatinib caproKit™ 50 reactions
1-1080-010	Dasatinib caproKit™ 10 reactions
1-1090-050	SAH caproKit™ 50 reactions
1-1090-010	SAH caproKit™ 10 reactions
1-1100-060	Biotin Capping Kit 60 reactions
1-8050-010	CCMS Starter Kit
1-8050-050	CCMS Starter Kit XL

The caproKit includes the respective Capture Compound, all buffers, reaction vials, protein positive control, competitor, and Streptavidin magnetic beads. CCMS Starter Kit includes caproBox™, caproMag™, Biotin Capping Kit, and three 10 reactions caproKits™ of choice. CCMS Starter Kit XL includes caproBox™, caproMag™, Biotin Capping Kit, and two 50 reactions caproKits™ of choice.

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Products & Services

CCMS technology is made available as ready to use caproKit reagents and services.

For more information please visit www.caprotec.com

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