



N²-GDP caproKit™ 50 Reactions

List of components

N²-GDP Capture Compound™

The N²-GDP caproKit™ allows a selective isolation of GDP- and GTP-binding proteins. The synthetic N²-GDP Capture Compound™ (Figure 1) uses guanosyl-diphosphate (GDP) as selectivity function to interrogate native proteins. GDP is attached via an aminobutyl group at N² to the Capture Compound Scaffold. The Capture Compound Mass Spectrometry (CCMS) technology enables analysis, discovery and characterization of GDP- and GTP-binding proteins through an efficient reduction of the complexity of the proteome.

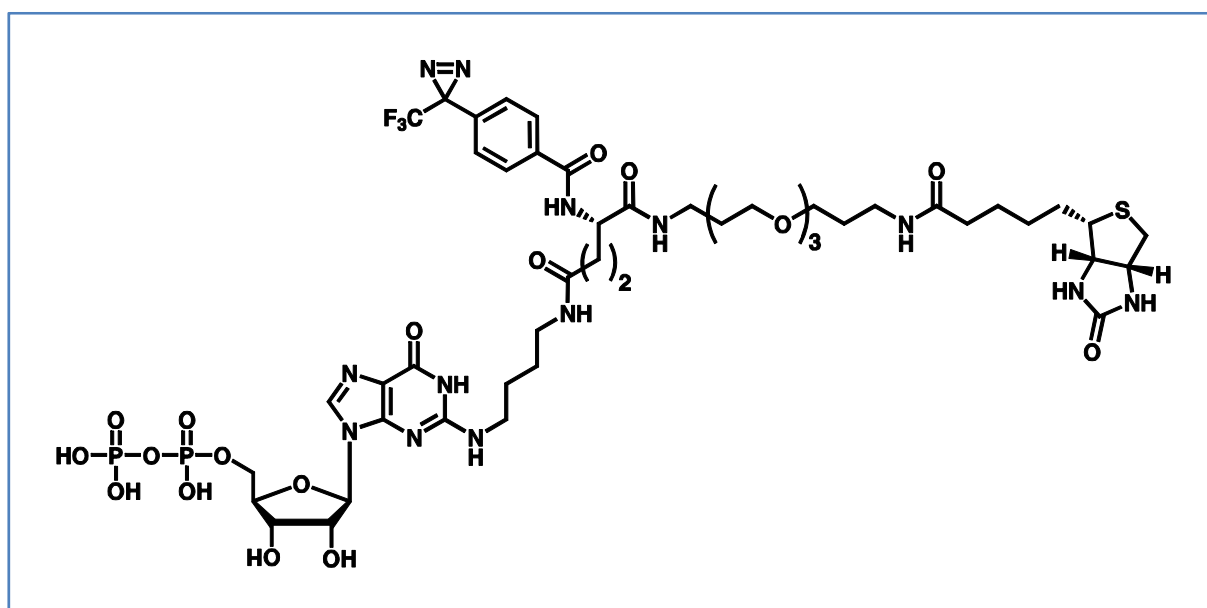


Figure 1: N²-GDP Capture Compound™ for the selective isolation of GDP- and GTP- binding proteins using GDP as selectivity function.

Item No	Component	Amount	Buffer composition
2-3010-050	Streptavidin coated magnetic beads (10 mg/ml SA-MB)	2.8 ml	Dynal Dynabeads MyOne™ Streptavidin C1 (Invitrogen)
2-2300-050	Capture buffer 2 (5x CB2)	1.2 ml	HEPES, KOAc, Glycerol, Triton X-100, pH 7.5
2-2200-050	Wash buffer 1 (5x WB1)	25 ml	Tris-HCl, EDTA, NaCl, Octyl-β-D-glucopyranoside, pH 7.9
2-6050-050	Magnesium chloride solution (1 M)	0.36 ml	MgCl ₂
2-6150-050	EDTA solution (100 mM)	0.12 ml	EDTA, pH 8.0
2-1051-050	N ² -GDP Capture Compound™ (N ² -GDP-CC, 100 μM)	0.6 ml	HEPES, pH 7.5
2-4050-050	GDP competitor (50 mM)	0.6 ml	HEPES, pH 7.5
2-5050-050	H-Ras (21.3 kDa, 23 μM)	10 μl	HEPES, NaCl, DTT, MgCl ₂ , GDP, Glycerol, pH 7.2
3-4011-000	12 PCR Tube strips 0.2 ml (AB-1114)	5	

Note: The H-Ras solution will not freeze due to the glycerol in the storage buffer. It is recommended to aliquot the N²-GDP Capture Compound™ solution upon arrival to avoid multiple freezing/thawing cycles of the stock solution. Protect the N²-GDP Capture Compound™ from direct light.

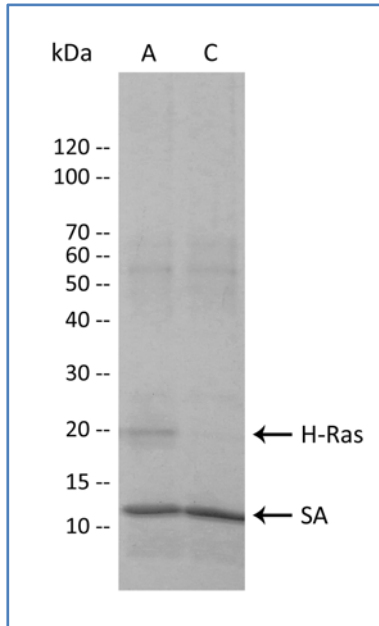
Separate 1.5 ml 5x WB1 in a fresh 2.0 ml tube for the assay. Dilute the rest of the 5x WB1 in a ratio of 1:5 in aqua bidest for washing steps and store at -20 °C to -18 °C. **Do not freeze the Streptavidin coated magnetic beads!** All solutions must be entirely thawed and mixed before usage.

Storage notification

Item No	Component	During shipment (max 3 days)	After receipt
2-3010-050	Streptavidin coated magnetic beads (10 mg/ml SA-MB)	4 to 8 °C	4 to 8 °C
2-2300-050	Capture buffer 2 (5x CB2)	4 to 8 °C	- 20 to -18 °C
2-2200-050	Wash buffer 1 (5x WB1)	4 to 8 °C	- 20 to -18 °C
2-6050-050	Magnesium chloride solution (1 M)	4 to 8 °C	- 20 to -18 °C
2-6150-050	EDTA solution (100 mM)	4 to 8 °C	- 20 to -18 °C
2-1051-050	N ² -GDP Capture Compound™ (N ² -GDP-CC, 100 μM)	4 to 8 °C	- 20 to -18 °C
2-4050-050	GDP competitor (50 mM)	4 to 8 °C	- 20 to -18 °C
2-5050-050	H-Ras (21.3 kDa, 23 μM)	4 to 8 °C	- 20 to -18 °C

Specified Functionality

Significant band (SDS-PAGE/silver stain) with 1.0 µg (46 pmol) of H-Ras and significant competition with GDP, when the capturing protocol described in the N²-GDP caproKit™ guideline is applied, caproBox™ and only kit components are used.



A: Capture assay with H-Ras

C: Control of "A" using 5 mM GDP as competitor

SA: Streptavidin from Streptavidin coated magnetic beads

Figure 2: Capture assay (A) and GDP competition control (C) of the positive control protein H-Ras analyzed by SDS-PAGE/silver stain.

Stability

The N²-GDP caproKit™ is stable under storage conditions for 6 months. After first use microbial contamination may occur.

Please read the material safety data sheet for this product at www.caprotec.com

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Head of Quality Control

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

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