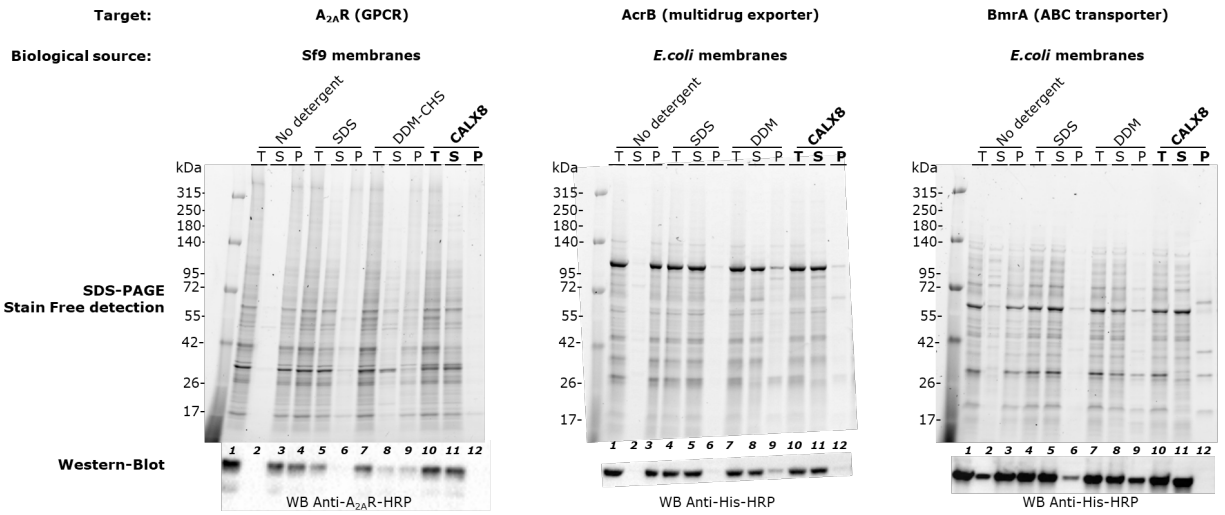
Technical Data Sheet – SOLUBILIZATION AND STABILIZATION SCREENING KIT

Biochemical Validation Data

Find below mainly biochemical validation data of some compounds of the solubilization and stabilization screening kit.

1. CALX8: Proprietary mild detergent

CALX8 solubilizes eukaryotic and prokaryotic membrane proteins with near total efficiency including GPCRs and transporters.



Membrane proteins solubilization by CALX8 reagent

The target was extracted from isolated membranes by using 20 mM CALX8. After solubilization, samples were centrifuged at 150,000 xg for 15 min. Proteins from pellets (P) and supernatants (S) were separated on BioRad 4 20% TGX gel by SDS-PAGE, transferred to LF-PVDF membrane and immunodetected with a specific antibody.

T= total, P= pellet, S= supernatant.

References

- Desuzinges Mandon E. et al. Novel systematic detergent screening method for membrane proteins solubilization. Anal Biochem. 2017 Jan 15 ; 517:40-49.

Full data sheet of CALX8

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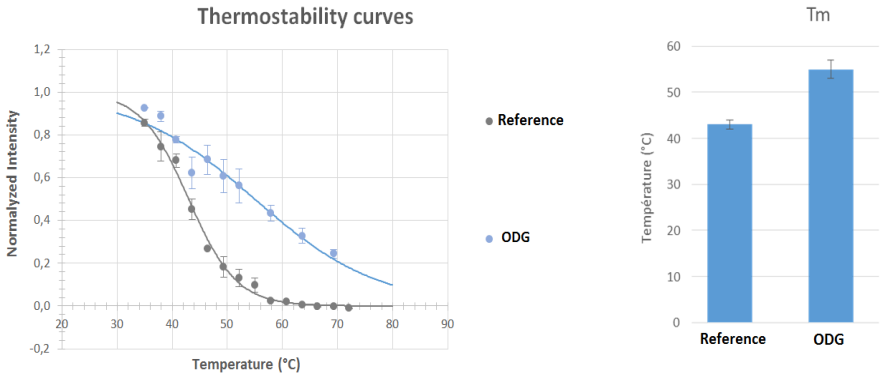
2. ODG: Proprietary mild detergent



Stabilization of GPCR target

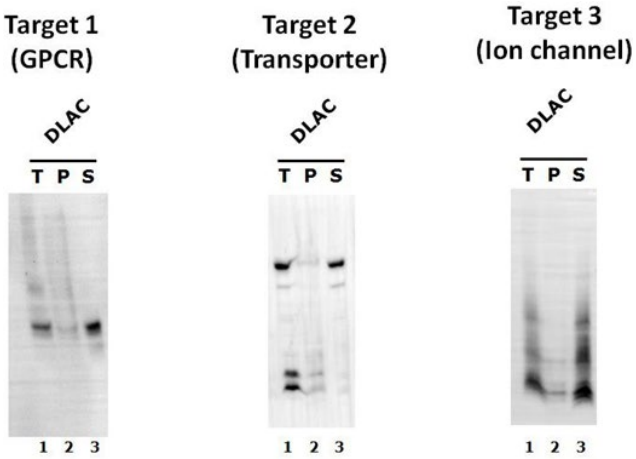
The GPCR protein was extracted using either reference detergent or ODG and heated at different temperatures for 30 min. After centrifugation at 20000g for 40 min, samples were separated on a 4-15% Tris-glycine SDS-PAGE, transferred to PVDF membrane and immunodetected with a specific antibody. Band intensity was measured and the resulting graph allowed Tm estimation.

References

- Lebaupain et al., Langmuir, 2006, 22 (21), pp 8881–8890

Full data sheet of ODG

3. DLAC : Soft proprietary detergen



Membrane proteins solubilization

The 3 targets were extracted from Sf9 membranes (GCPR), E. coli membranes (Transporter) and mammalian membranes (ion channel) by using DLAC reagent at 10-fold the critical micelle concentration (cmc). After solubilization, samples were centrifuged at 100000g. Proteins from pellets (P) and supernatants (S) were separated on a 4-15% Tris-glycine SDS-PAGE, transferred to PVDF membrane and immunodetected with a specific antibody

T = total, P = pellet, S = supernatant.

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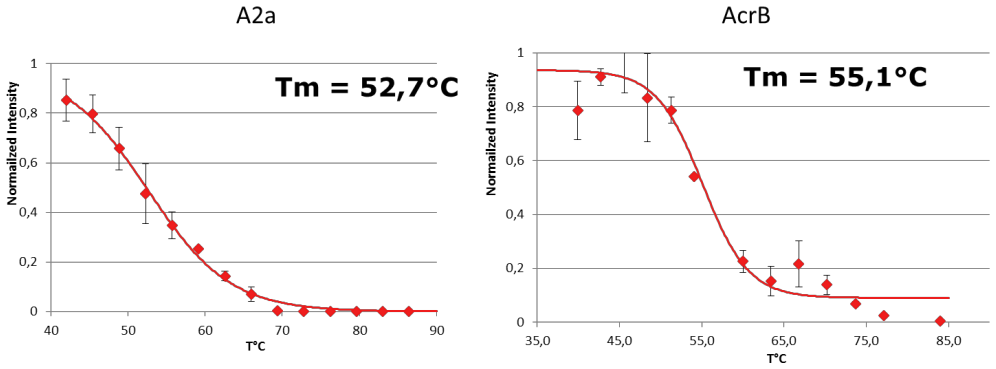
- Lebaupain et al., Langmuir, 2006, 22 (21), pp 8881–8890

Full data sheet of DLAC

4. FLAC6 : Soft proprietary detergent

Thermalshift assay on membranes solubilized with 15- fold FLAC6 cmc

Unfolding of either A2a receptor or AcrB was followed by western blotting after applying a temperature gradient and high-speed centrifugation.



References

- Lebaupain et al., Langmuir, 2006, 22 (21), pp 8881–8890

Full data sheet of FLAC6

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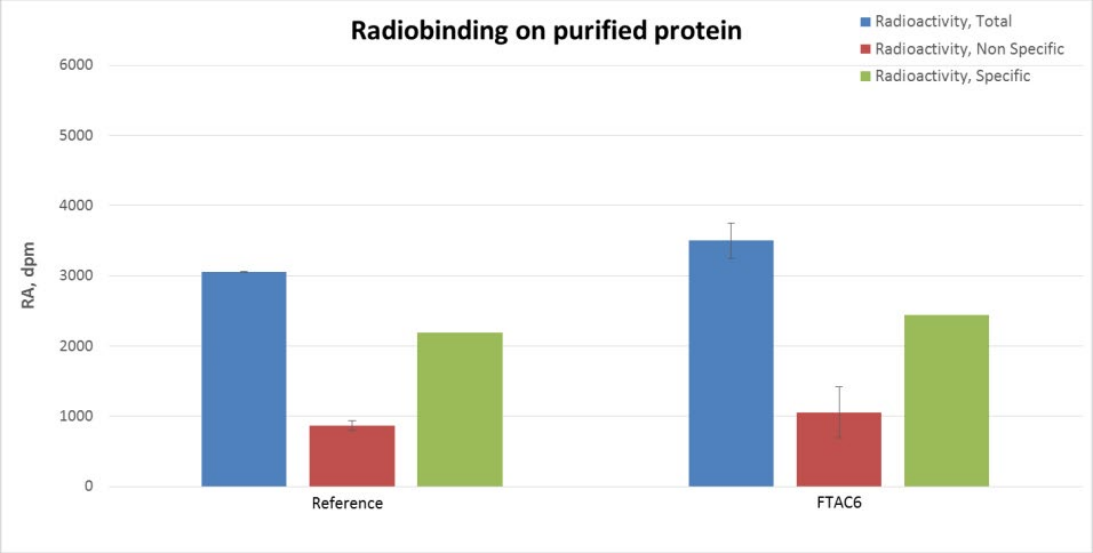
5. FTAC6: Soft proprietary stabilization reagent



Binding of radioligand on GPCR protein, purified in reference detergent with or without addition of FTAC6 as an additive

Purified protein was incubated with radioligand in absence (total, blue bars) or presence (Non Specific signal, red bars) of an excess of cold ligand. After filtration on GF/C membranes and washing, scintillation agent was added and radioactivity was detected using a Microbeta2. Specific radioactivity (green bars) corresponds to (total signal) – (non-specific signal).

References

- Damian, M., S. Perino, A. Polidori, A. Martin, L. Serre, B. Pucci and J.-L. Baneres (2007). "New tensio-active molecules stabilize a human G protein-coupledreceptor in solution." Febs Letters **581**(10): 1944-1950.
- Park, K.-H., C. Berrier, F. Lebaupain, B. Pucci, J.-L. Popot, A. Ghazi and F. Zito(2007). "Fluorinated and hemifluorinated surfactants as alternatives to detergents for membrane protein cell-free synthesis." Biochemical Journal **403**: 183-187.
- Joubert, O., R. Nehme, D. Fleury, M. De Rivoyre, M. Bidet, A. Polidori, M. Ruat,
- B. Pucci, P. Mollat and I. Mus-Veteau (2009). "Functional studies of membrane-bound and purified human Hedgehog receptor Patched expressed in yeast." Biochimica Et Biophysica Acta-Biomembranes **1788**(9): 1813-1821.

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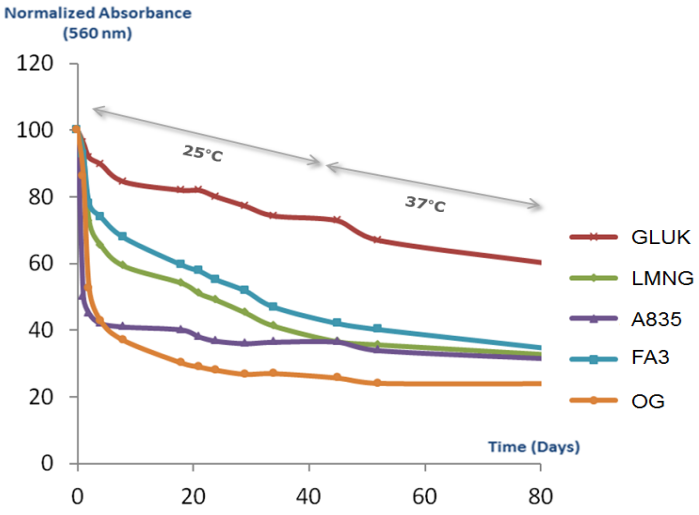
- Nehme, R., O. Joubert, M. Bidet, B. Lacombe, A. Polidori, B. Pucci and I. Mus- Veteau (2010). "Stability study of the human G-protein coupled receptor, Smoothened." Biochimica Et Biophysica Acta-Biomembranes **1798**(6): 1100-1110.5- Park, K.-H., E. Billon-Denis, T. Dahmane, F. Lebaupain, B. Pucci, C. Breyton and F. Zito (2011). "In the cauldron of cell-free synthesis of membrane proteins: playing with new surfactants." New *Biotechnology* ***28****(3): 255-261.*

Full data sheet of FTAC6

6. CALXGLUK: Soft proprietary stabilization reagent



Stabilization of the Bacteriorhodopsin

After solubilization and purification in OG, reagents were added as stabilizers.

Bacteriorhodopsin's activity was monitored over time at first 25°C and then 37°C by measuring absorbance at 560nm.

References

- Proprietary patent: "Synthesis of amphiphilic calixarene glycoside detergents and use of same for extracting and stabilizing native functional membrane proteins"; WO 2015158575 A1 ; Publication date: Oct 22, 2015.

Full data sheet of CALXGLUK

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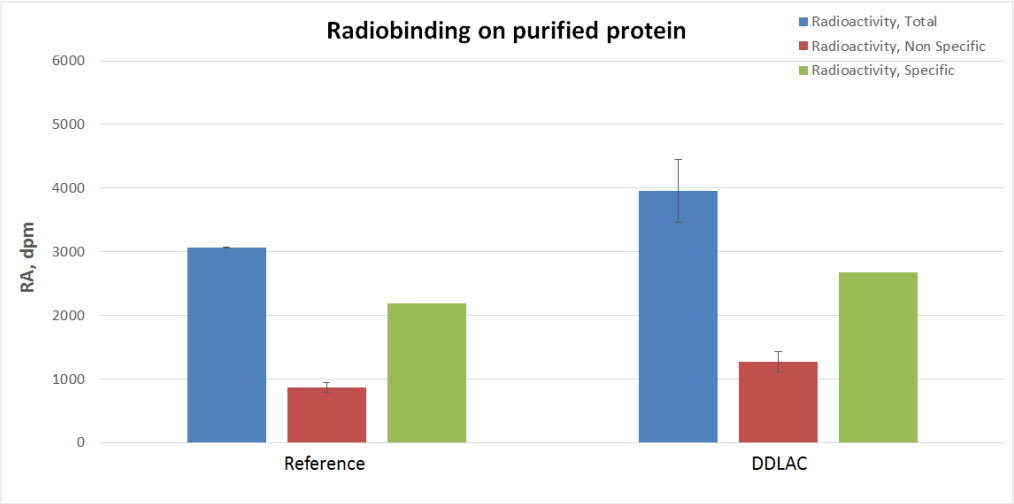
7. DDLAC: Soft proprietary stabilization reagent



Binding of radioligand on GPCR protein, purified in reference detergent with or without addition of DDLAC as an additive

Purified protein was incubated with radioligand in absence (total, blue bars) or presence (Non-Specific signal, red bars) of an excess of cold ligand. After filtration on GF/C membranes and washing, scintillation agent was added, and radioactivity was detected using a Microbeta2. Specific radioactivity (green bars) corresponds to (total signal) – (non-specific signal).

References

- Lebaupain et al., Langmuir, 2006, 22 (21), pp 8881–8890

Full data sheet of DDLAC

All data sheets of reagents included in the kit are available on our website:

- DDTAC data sheet : soft proprietary detergent
- DDG data sheet : soft proprietary detergent
- FTAC8 data sheet : soft proprietary stabilization reagent