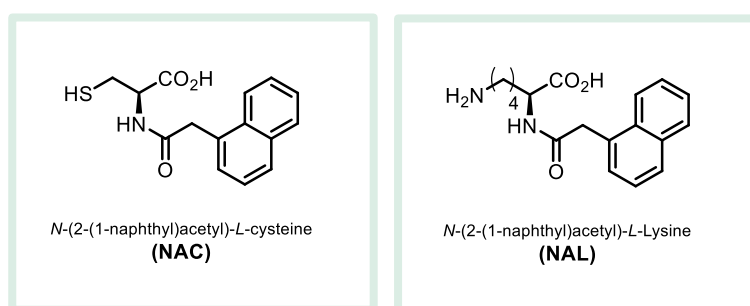


Non-animal method for predicting skin sensitization (OECD TG442C)

ADRA kit

ADRA (**A**mino acid **D**erivative **R**eactivity **A**ssay) is a *in chemico* test method, representing an alternative (non-animal) method for the evaluation of skin sensitization compounds. The kit comprises two amino acid derivatives, NAC (N-(2-(1-naphthyl)acetyl)-L-cysteine) and NAL (α -N-(2-(1-naphthyl)acetyl)-L-lysine), which are composed out of a nucleophilic region and a detection molecule (naphthalene rings).

ADRA is proposed to address the molecular initiating event of skin sensitization AOP (**A**dverse **O**utcome **P**athway) by quantifying the reactivity of the test compounds against the model synthetic amino acid derivatives. The reaction is monitored by HPLC-analysis after 24 hours incubation at 25°C and 281 nm, determining the relative residual concentration of NAC and NAL in the reaction liquid.



Advantages of ADRA

- NAC and NAL are detected at a relatively long wavelength (281 nm).
 - Preventing co-elution of the test chemical and the nucleophilic reagent.
- ADRA avoids precipitation by using only 1% of reactants of the existing method.
- Variability of result values are very low.
 - In ADRA, the reaction is stopped by the addition of a fixing solution (2.5%TFA) before analyzing
- Multiple test chemicals can be assayed in a short period of time.
 - The test procedure is performed using a single 96-well plate and a multichannel pipette.


Kit Reagents



Components	Amount
NAC	2 vials × 10 ml
NAL	2 vials × 10 ml
NAC Buffer (pH 8.0) premixed	2 vials × 300 ml
NAL Buffer (pH 10.2) premixed	2 vials × 300 ml
0.01 mol/l EDTA Solution	2 vials × 1 ml

Code No.	Product Name	Package Size
296-80901	ADRA Kit	1 Kit

Additional reagents required

Code No.	Product Name		Package Size
204-02743	Trifluoroacetic Acid		25 ml
015-08633	Acetonitrile		3 l
217-01031	Ultrapure Water *		1 l
016-00346	Acetone **		500 ml
043-07216	Dimethyl Sulfoxide **		500 ml

* Use water with low metal content.

** Not to be used, if the test chemical dissolves in water or acetonitrile.

Phenylacetaldehyde (CAS RN® 122-78-1) is required as a positive control.

Apparatus required

- 1) Electronic balance – precision scale: ± 0.1 mg
- 2) Three Micropipettes – 10 μ l, 100 μ l and 1000 μ l
- 3) 12 channel pipette – 150 μ l
- 4) HPLC system – light-shielding auto-sampler for 96-well plates and 0.3 ml/min liquid feeding
- 5) UV detector – photodiode array (PDA) detector or absorbance detector (281 nm)
- 6) HPLC column
- 7) pH meter – precision scale: ± 0.01 pH
- 8) Incubator – 25 °C
- 9) 96-well plates
- 10) 500 mL plastic bottles
- 11) Vortex mixer
- 12) Plate seal*
* Use the seal having high sealability and solvent-resistant performance.
- 13) Plate shaker
- 14) Plate centrifuge

Note: to avoid NAC/NAL dimerization upon metal ion contamination, all consumables (except for components for HPLC analysis) must be made of polypropylene or polyethylene.

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