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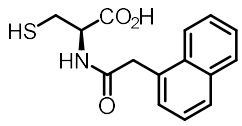
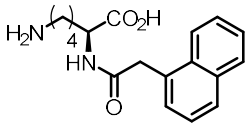
Non-animal method for predicting skin sensitization (OECD TG442C)

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 derivates, NAC (N-(2-(1- **n**aphthyl)**a**cetyl)-L-**c**ysteine) and NAL (α-N-(2-(1-**n**aphthyl)**a**cetyl)-L-**l**ysine), 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.



N-(2-(1-naphthyl)acetyl)-*L*-cysteine
(NAC)



N-(2-(1-naphthyl)acetyl)-*L*-Lysine
(NAL)

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

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

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Additional reagents required

Code No.	Product Name	Package Size
204-02743	Trifluoroacetic Acid Ref	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 µl, 100 µl and 1000 µl
3) 12 channel pipette – 150 µl
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 ℃
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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