Human Cell Line Activation Test (h-CLAT)

In Vitro Skin Sensitisation Assay

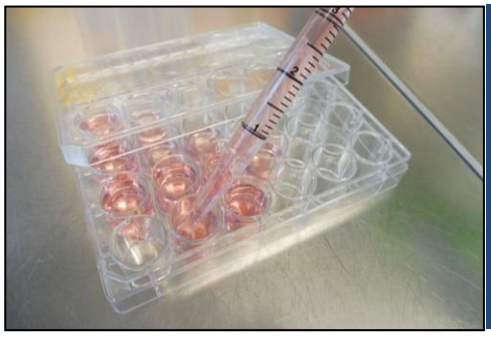
The *in vitro* h-CLAT addresses the third molecular key event of the adverse outcome pathway (AOP) of skin sensitization.[1] The UN GHS (United Nations Globally Harmonized System of Classification and Labelling of Chemicals) defines a skin sensitiser as a substance that will cause an allergic response after skin contact.[2]

The h-CLAT is validated by the EURL ECVAM (European Union Reference Laboratory for Alternatives to Animal Testing) and is performed in accordance with the OECD guidance OECD 442E at Eurofins BioPharma Product Testing Munich GmbH [1, 3] with chemicals, cosmetics or personal care products and pharmaceuticals.

The h-CLAT is one of three test methods (DPRA and KeratinoSens™) for the assessment of skin sensitisation potential.

Assessment of Skin Sensitisation Potential with the h-CLAT

- The third molecular key event of skin sensitisation addresses the activation of dendritic cells (DC), which is typically accompanied by expression of specific cell surface markers, chemokines and cytokines.
- The surface molecules CD86 and CD54 are typical markers of DC activation and play a critical role in T-cell priming by DCs, activating an immune response.
- The human monocytic leukemia cell line (THP-1) is used to quantify the expression of the two cell surface markers CD86 and CD54 using fluorescence-activated cell sorting (FACS). The changes in the expression level reflect the activation of DCs triggered by sensitisers.
- The cytotoxicity is also measured to conduct whether the upregulation of the surface markers CD86 and CD54 occurs at sub-cytotoxic concentrations.



"The third key event is the activation of dendritic cells (DC), typically assessed by expression of specific cell surface markers. These surface molecules CD86 and CD54 are typical markers of monocytic THP-1 cell activation and play a critical role in T-cell priming." [1] The elevation is detected with fluorescence-activated cell sorting (FACS) of the two markers CD86 and CD54.

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Procedure

Principle of the h-CLAT

Protocol	
Cell line	Human monocytic leukemia cell line (THP-1)
Analysis	Expression levels of surface markers CD86 and CD54 measured by FACS with fluorochrome-tagged antibodies Cell viability determination
Concentrations	8 stock solutions based on the CV75 (cell viability of 75%) value or 500 mg/mL
Exposure time	24 h
Quality controls	Positive control: 2,4-dinitrochlorobenzene (4 µg/mL) Solvent controls: 1% 0.9% NaCl, 0.2% dimethylsulfoxide or tetrahydrofuran
Solvents of test chemical	0.9% NaCl Dimethylsulfoxid Tetrahydrofuran
Application	Dose finding assay (determination of CV75 value) Two independent performed main experiments Equivocal results require a third repetition
Data delivery	Relative fluorescence intensity (RFI) of CD86 and CD54 Cell viability
Positive prediction	RFI of CD86 ≥ 150% at any tested concentration with cell viability ≥ 50% and/or RFI of CD54 ≥ 200% at any tested concentration with cell viability ≥ 50%

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Data

Eurofins Data for demonstrating technical proficiency of the h-CLAT

Chemical	CV75 (OECD)	Prediction (OECD)	CV75 (EF)	HV1 (EF)	HV2 (EF)	HV3 (EF)	Prediction (EF)
Non-sensitising Chemicals							
Glycerol	>5000	**Negative**	>1000	CD86 - / CD54 -	CD86 - / CD54 -	--	**Negative**
Lactic acid	>5000	**Negative**	>1000	CD86 - / CD54 -	CD86 - / CD54 -	--	**Negative**
Vanillin	n.a.	**Negative**	542.63	CD86 - / CD54 -	CD86 - / CD54 -	--	**Negative**
1-Butanol	n.a.	**Negative**	>1000	CD86 - / CD54 -	CD86 - / CD54 -	--	**Negative**
Sensitising Chemicals							
2,4-Dinitrochlorobenzene	2-12	**Positive**	3.75	CD86 + / CD54 +	CD86 + / CD54 +	--	**Positive**
Nickelsulfate	30-500	**Positive**	230.19	CD86 + / CD54 +	CD86 + / CD54 +	--	**Positive**
Imidazolidinyl Urea	25-100	**Positive**	38.8	CD86 + / CD54 +	CD86 + / CD54 +	--	**Positive**
Chloramin T	n.a.	**Positive**	336.54	CD86 - / CD54 -	CD86 + / CD54 +	CD86 - / CD54 +	**Positive**
Hydroxy-citronellal	n.a.	**Positive**	650.64	CD86 + / CD54 +	CD86 - / CD54 -	CD86 + / CD54 -	**Positive**

n.a.: not specified in the guideline EF = Eurofins Munich GmbH

Table 1: Eurofins h-CLAT data of nine tested proficiency chemicals compared to the data of the OECD guideline[1].

In Table 1 the obtained data from the h-CLAT of four non-sensitising and five sensitising chemicals are shown. The prediction of all tested chemicals was correct in comparison to the classification of the OECD guideline.

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References

1) OECD Guidelines for Testing of Chemicals, No. 442E: “In Vitro Skin Sensitisation assays addressing the Key Event on activation of dendritic cells on the Adverse Outcome Pathway for Skin Sensitisation”, adopted 09 October 2017.

2) UN (2015), United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Sixth revised edition, UN New York and Geneva.

3) EC EURL-ECVAM (2015). Recommendation on the human Cell Line Activation Test (h-CLAT) for skin sensitisation testing.