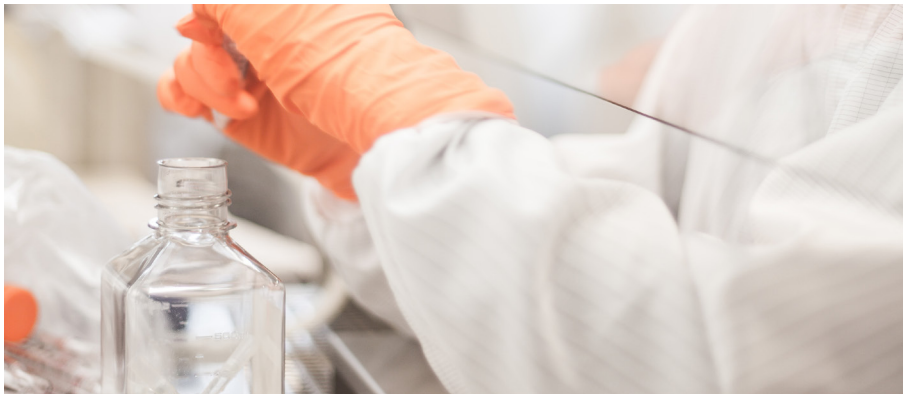


IN VITRO RESPIRATORY SENSITIZATION TEST

GARD™air—for safer products and identification of workplace hazards

GARD™air is the first assay capable of predicting the potential of chemicals to induce allergic hypersensitivity reactions in airways. The assay supports the development of safer products and contributes to a less harmful working environment, through early and reliable hazard identification of potential respiratory allergens in occupational settings.



GARD™air provides a robust solution to the demands for 3Rs by generating reliable outcomes via *in vitro* testing using a proprietary human cell line (SenzaCell™).

Why should I use the assay?

Exposure to respiratory sensitizers from consumer products or in the workplace may result in the development of allergic asthma, a serious and irreversible disease with a major impact on individuals affected. GARD™air identifies respiratory sensitizers early on and during the entire product development. This means that the assay can be used in pre-product screening to both prevent the release of hazardous substances into the consumer market and ensure appropriate preventive exposure controls at work.

How to GARD your products in six steps:

- 1 **GARD Input Finder:** Dose-response to find the GARD input concentration where 90% of SenzaCells™ survive.
- 2 **GARD Main Stimulation:** SenzaCells™ are exposed at the GARD input concentration.
- 3 **RNA extraction:** Following 24h of cellular stimulation, total RNA is isolated using an RNA extraction kit.
- 4 **Gene expression profiling:** Gene expression of the 28 genes in the biomarker signature is measured using NanoString.
- 5 **GARD data analysis application:** The resulting gene expression is analysed by pattern recognition, using a machine learning algorithm based on a fixed set of reference samples.
- 6 **Results:** Report provided on test substance classification as sensitizer or non-sensitizer.

AT A GLANCE

Tox Endpoint: Specific identification of respiratory sensitizing chemicals.

Parameters: Genomic readout of 28 genes relevant to respiratory sensitization.

Why: Develop safer products and offer employees a less hazardous working environment.

How: State-of-the-art machine learning to compare patterns of gene expressions.

Performance: ~ 90% accuracy on in-house validated data.

Turnaround time: 4-6 weeks.

Sample requirements: 0.5 g (solids) or 1 ml (liquids). Can be adapted to lower amounts.

ABOUT SENZAGEN

SenzaGen is dedicated to the development of innovative *in vitro* methods for safety testing of various toxicological endpoints across different industries. SenzaGen performs GARD™ in its own laboratory and through CRO partners around the world.

EU supported development

GARD™air is supported by the EU research initiative Horizon 2020 and was selected as a flagship project within health in 2019. Grant agreement No 756014.



Human relevance, genomics and machine learning

Development of respiratory sensitization is dependent upon activation of both innate and adaptive immune responses. Key mechanisms involve activation of dendritic cells and polarization of the T-cell response towards a Th2 immune profile.

GARD™air utilizes a combination of relevant human cells, genomics and machine learning to mimic this immune response *in vitro*. The assay is based on a human dendritic-like cell line (SenzaCells™) and uses state-of-the-art machine learning to classify chemicals by monitoring the expression of 28 genes involved in cellular pathways associated with respiratory sensitization.

Results from an in-house study of two external test sets. Accuracy: 91%, sensitivity 92% and specificity 90%. Moreover, 88% of the skin sensitizers were correctly classified as non-respiratory sensitizers.

Chemical Entity	True group	Test 1	Test 2
Chloramine-T hydrate	RS	RS	RS
Isophorone diisocyanate	RS	RS	-
Maleic anhydride	RS	-	RS
Phenyl isocyanate (MDI)	RS	-	RS
Phthalic anhydride	RS	-	RS
Piperazine	RS	RS	RS
Reactive orange 16	RS	RS	RS
Toluene diisocyanate	RS	-	NRS
Trimelitic anhydride	RS	-	RS
2-Mercaptobenzothiazole	SS/NRS	NRS	-
Cinnamyl alcohol	SS/NRS	NRS	-
DNCB	SS/NRS	NRS	NRS
Eugenol	SS/NRS	NRS	-
Glyoxal	SS/NRS	RS	-
Isoeugenol	SS/NRS	NRS	-
PPD	SS/NRS	NRS	NRS
Resorcinol	SS/NRS	NRS	-
4-Hydroxybenzoic acid	NRS	NRS	-
Benzaldehyde	NRS	NRS	-
Chlorobenzene	NRS	-	NRS
Diethyl phthalate	NRS	NRS	-
DMSO	NRS	-	NRS
Glycerol	NRS	NRS	-
Octanoic acid	NRS	NRS	-
Phenol	NRS	NRS	-
Salicylic acid	NRS	RS	-
SDS	NRS	NRS	-

RS: Respiratory sensitizer; SS: Skin sensitizer; NRS: Non-respiratory sensitizer; NS: Non-sensitizer

FEATURES AND BENEFITS

Relevant human cell type

- SenzaCells™: Human dendritic-like cell line.

Test chemical solvents

- DMSO and H₂O within standard protocols. Several alternative solvents available upon request.
- Also applicable for samples with low water solubility.

Analysis

- Gene expression measurements of 28 genomic biomarkers in the GARD™ Respiratory Prediction Signature (GRPS).

- Covers specific mechanistic pathways – avoids false positive classifications of skin sensitizers.

Classifications

- Cloud-based prediction model based on machine learning correlates gene expression to sensitizing potential.
- Transparent classification; no subjective judgement required.

Performance

- ~90% accuracy on in-house validation data.

Time and cost

- Fast and reliable results during product development.
- Quick turnaround time: 4-6 weeks to final report.

Compatibility



In combination with GARD™skin and GARD™potency for hazard assessment of both skin and respiratory sensitizers.

*Validation study, OECD Test Guideline Program (TGP no. 4.106). Johansson H. et al., Toxicological Sciences 2019.

SENZAGEN

THE PREDICTION SIGNATURE

Genes from relevant pathways including:

-  Innate immune activation
-  T-cell polarization to Th2

AVAILABLE ASSAYS

GARD™skin: *In vitro* classification of skin sensitizers with 94% accuracy.*

GARD™potency: First-in-class *in vitro* assay for CLP/GHS classification of skin sensitizers.

GARD™air: First-in-class *in vitro* assay for specific identification of respiratory sensitizing chemicals.

GARD™skin Medical Device: *In vitro* classification of skin sensitizers in polar and non-polar extraction vehicles.