IN VITRO SKIN SENSITIZATION POTENCY TEST

GARD™potency
for GHS/CLP classification

GARD™potency is the first *in vitro* assay that allows for potency sub-categorization of confirmed skin sensitizers according to the GHS/CLP system. The assay is included in the OECD Test Guideline Programme (TGP 4.106) and is suitable for submissions under REACH. By providing reliable and accurate potency data, GARD™potency is also a useful tool for product hazard labeling and for non-regulatory stages of product development.

The GARD™potency assay provides a solution to the demands for 3Rs by generating reliable classifications on skin sensitizing potency to support in regulatory decision-making, reducing the need for confirmatory *in vivo* studies.

Why should I use the assay?

Chemical regulatory authorities around the globe increasingly demand testing data for skin sensitization hazard and potency classification. Currently accepted OECD *in vitro* methods for skin sensitization only allow for hazard identification, and potency classification often necessitates confirmatory animal studies. GARD™potency is the first *in vitro* assay specifically designed to meet regulatory requirements in the REACH registration for sub-categorization of skin sensitizers according to the GHS/CLP system (1A strong, 1B weak). Data generated from the assay enable accurate product hazard labeling to alert workers and consumers of potential dangers, and facilitate industrial compliance with regulatory agencies standard information requirements for the endpoint of skin sensitization.

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 51 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 strong sensitizer or weak sensitizer as defined by the GHS/CLP system.

AT A GLANCE

Tox Endpoint: Skin sensitizing potency sub-categorization according to GHS/CLP (1A: strong-, 1B: weak-sensitizer).

Parameters: Genomic readout of 51 genes relevant to skin sensitization.

Why: Develop safer products, comply with regulatory information requirements, and enable product hazard labeling according to GHS/CLP.

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

Performance: 82% accuracy for hazard assessment and GHS/CLP potency sub-categorization reported in an OECD validation study.

Test time: 2 weeks.

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

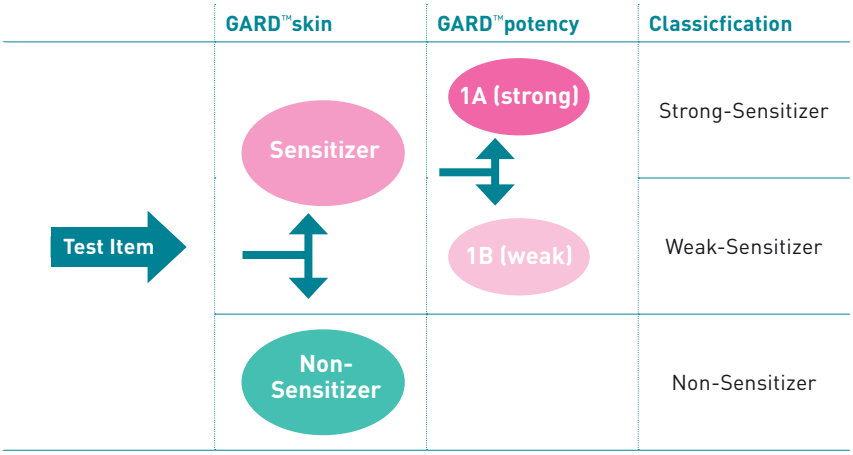
ABOUT SENZAGEN

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.

Human relevance, genomics and machine learning

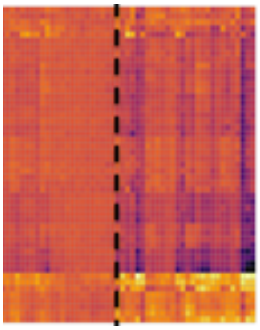
Development of skin sensitization is dependent upon activation of both innate and adaptive immune responses. Key mechanisms involve activation of dendritic cells and induction of an allergen specific T-cell response.

GARD™potency utilizes a combination of human relevant 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. It sub-categorizes skin sensitizers according to the GHS/CLP system by monitoring the expression of 51 genes specifically regulated if the sensitizing potency of a test chemical is sufficiently strong.



Potency assessments are performed using a tiered approach including both the GARD™skin and the GARD™potency assays. In a first tier, a test item is classified in GARD™skin as either skin sensitizer or non-sensitizer. In a second tier, any test item classified as a sensitizer in the first tier is further classified in GARD™potency for sub-categorization according to the CLP system.

THE PREDICTION SIGNATURE



1B Weak 1A Strong

The 51 genes in the signature are differentially regulated depending on whether SenzaCells™ are exposed to a strong (1A) or a weak (1B) sensitizer. This information is used for potency sub-categorization of unknown test items.

AVAILABLE ASSAYS

- GARD™skin
- GARD™potency
- GARD™air
- GARD™skin Medical Device

FEATURES AND BENEFITS

Relevant human cell type

- SenzaCells™: Human dendritic-like cell line.

Solvents

- DMSO and H_2O within standard protocols. Several alternative solvents available upon request.
- Applicable also for samples with low water solubility.

Testing strategy

- Potency assessments are performed using a tiered approach:
  - GARD™skin for identification of sensitization hazard.
  - GARD™potency for sub-categorization of confirmed sensitizers according to the GHS/CLP system.

Analysis

- Gene expression measurement of 51 genomic biomarkers in GARD™ Potency Prediction Signature (GPPS).

Classifications

- Cloud-based prediction model based on machine learning correlates gene expression to sensitizing potency according to the GHS/CLP system.
- Transparent classification; no subjective judgement required.

Performance

- 82% accuracy based on data from a validation study performed in compliance with OECD guidelines.

Time and cost

- Fast and reliable results during product development.
- Test time: 2 weeks.

Regulatory compliance

- GARD™potency is currently under EURL ECVAM validation and included in the OECD Test Guideline Program (TGP 4.106).
- GARD™potency results can already now be used in REACH dossiers in a weight of evidence approach to meet data requirements under the REACH Regulation 2016/1688 (REACH annex XI).
- GARD™potency sub-categorizes chemicals according to the CLP system as specified in Regulation (EC) No. 1272/2008 on classification, labelling and packaging of substances and mixtures.

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