IN VITRO SKIN SENSITIZATION TEST

GARD™skin Medical Device
—human relevant data for safer products

GARD™skin Medical Device classifies leachables in extracts of Medical Devices as either skin sensitizers or non-sensitizers and can be used for skin sensitization testing in the Biological Evaluation of Medical Devices according to ISO 10993.

GARD™skin Medical Device can be used for testing of skin sensitization with extraction vehicles according to ISO 10993-12:2012.

Why should I use the assay?

GARD™skin Medical Device provides a robust solution to the demands for 3R by generating reliable outcomes via *in vitro* testing. The assay is based on a human dendritic-like cell line (SenzaCells™) and classifies leachables in extracts as either skin sensitizers or non-sensitizers, based on the readout from a genomic biomarker signature. The large informational content of this approach provides a more holistic view of the immunological response to skin sensitizing chemicals, which is reflected by its high predictive accuracy shown for neat chemicals.

Extraction vehicles according to ISO 10993-12

GARD™skin Medical Device can be used with polar and non-polar extraction vehicles according to ISO10993-12:2012 (saline, sesame oil, Super Refined Olive Oil and cell culture media). The assay is also compatible with solvents already validated in GARD™skin.

How to GARD your products in six steps:

1. **GARD Input Finder:** Dose-response to find the GARD input concentration of the polar and non-polar extracts of the material 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 200 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 sample classification as sensitizer or non-sensitizer.

AT A GLANCE

Tox Endpoint: Specific identification of skin sensitizing chemicals.

Parameters: Genomic readout of 200 genes relevant to skin sensitization.

How: State-of-the-art machine learning tools used to develop the model. Transparent classification; no subjective judgement required.

Why: Develop safer products based on human relevant data supporting the principles of 3R.

Extraction vehicles: Saline, sesame oil, Super Refined Olive Oil, cell culture media.

Performance: GARD™skin; Accuracy 94%, Sensitivity 93% and Specificity 96%.*

Test time: 2 weeks.

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.

Detection of leachables in polar and non-polar extracts vehicles

The ability to detect leachables from Medical Devices in saline and oil was shown using model materials spiked with strong, moderate and weak sensitizers. Three additional medical grade materials were used as control materials. Extracts from the materials were prepared according to ISO 10993-12:2012; 0.2 g/ml, 37 ± 1 ° C for 72 ± 2 h. All model materials were correctly classified, the spiked materials as sensitizers, and the non-spiked materials as non-sensitizers. The three medical grade materials were all classified as non-sensitizers.

Material	Chemicals	Sensitizing potential		GARD™skin Medical Device		
		LLNA	HP	Saline	Olive oil	Sesame oil
Silicone	none	N/A	N/A	NS	NS	NS
	2-aminophenol	Strong	cat 2	S	S	S
	cinnamic aldehyde	Moderate	cat 2	S	S	S
	propyl gallate	Strong	cat 2	S	S	S
	phenyl benzoate	Weak	cat 3	S	S	S
TPU	none	N/A	N/A	NS	NS	-
	propyl gallate	Strong	cat 2	S	S	-
	phenyl benzoate	Weak	cat 3	S	S	-
Silicone tube	-	N/A	N/A	NS	NS	NS
TPU tube	-	N/A	N/A	NS	NS	NS
PVC tube	-	N/A	N/A	NS	NS	NS

Results from an in-house validation study testing of model materials with GARD™skin Medical Device. Sensitizing potential for LLNA (as listed in the CE STTF database) and Human potency classification (HP) for the chemicals (Basketter et al. 2014). NS: non-sensitizer, S: Sensitizer, -: not tested.

SUITABLE FOR

- Medical Device classified products
- Medical grade materials

THE PREDICTION SIGNATURE

Recognition of foreign substances e.g. TLRs, RXR, AGR

Immunological self-defence mechanisms e.g. CD80, CD86

Cellular stress responses e.g. NRF2-pathway

Communication e.g. chemotaxis receptors

AVAILABLE ASSAYS

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

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.

FEATURES AND BENEFITS

Relevant human cell type

- SenzaCells™: Human dendritic-like cell line.

Extraction vehicles

- Polar and non-polar extraction vehicles as described in ISO10993-12:2012 (saline, Super Refined Olive Oil, sesame oil, cell culture media).

Analysis

- Gene expression measurements of 200 genomic biomarkers in GARD™skin Prediction Signature (GPS). Covers specific mechanistic pathways.

Classifications

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

Performance

- Same prediction signature as GARD™skin. GARD™skin classifies neat chemicals with 94% accuracy.*

Time and cost

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

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

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