ESCO® ASTER

COSMETIC and COSMECEUTICAL TESTING SERVICES

ABOUT US

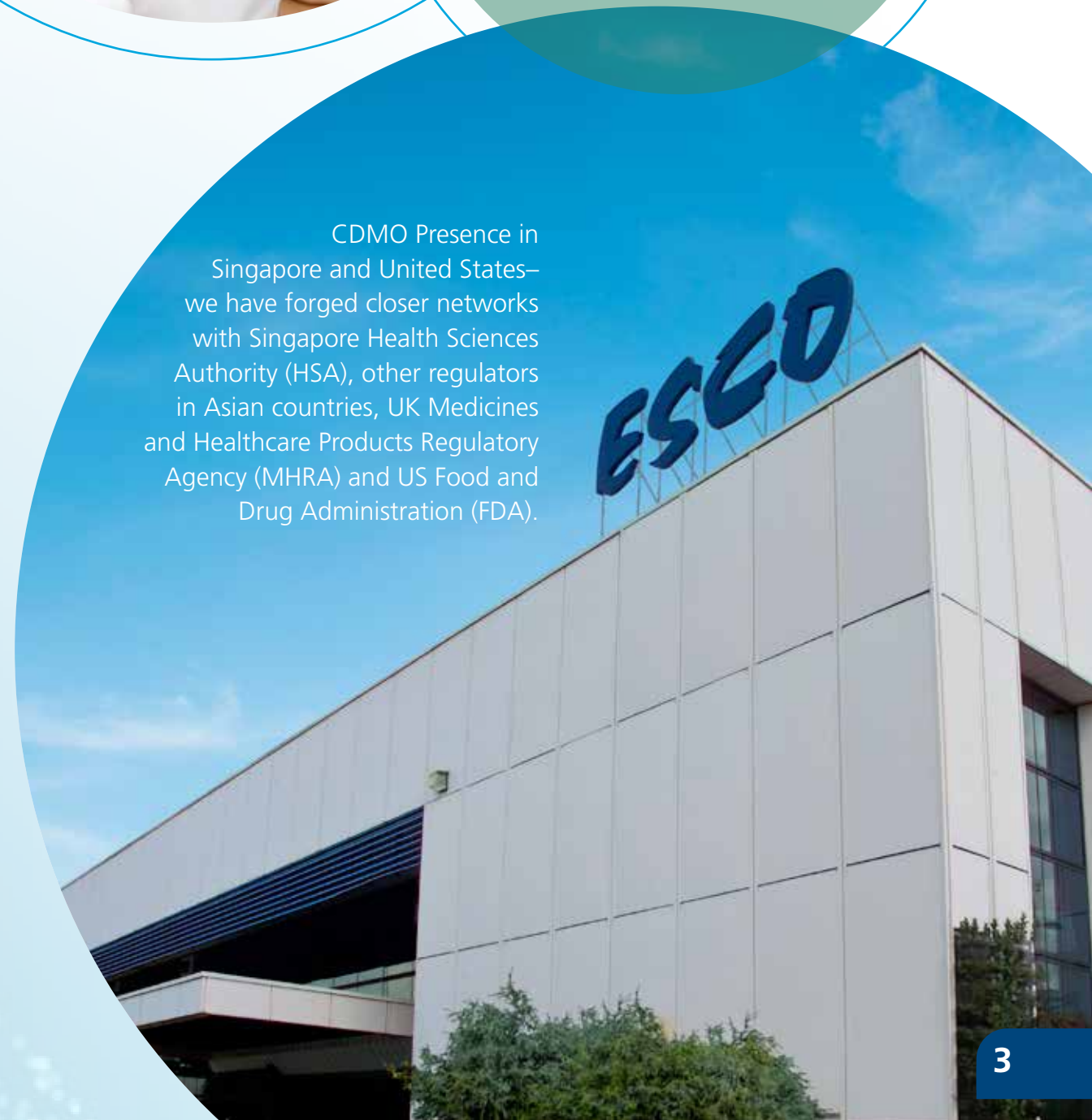
Esco Aster is a contract development and manufacturing organisation (CDMO) spun out as an independent subsidiary in 2017 from Esco Lifesciences Group founded in 1978. It aims to bridge non-GMP laboratory processes into GLP/cGMP, offers process development services of bench scale into cGMP scale for CMC package; leading to Investigational New Drug (IND) Phase I and II.

As a leading company in cell culture, we are also supporting European Centre for the Validation of Alternative Methods for Skin Testing

We provide *in vitro* support for skin safety testing and claim support from our laboratory.

We have a diverse network of investigators including dermatologists, dentists, medical doctors, laboratory scientists/ technologists, biochemists and statisticians, regularly working with brand leaders worldwide to provide human clinical trial studies

CDMO Presence in Singapore and United States– we have forged closer networks with Singapore Health Sciences Authority (HSA), other regulators in Asian countries, UK Medicines and Healthcare Products Regulatory Agency (MHRA) and US Food and Drug Administration (FDA).



DISCOVERY "FROM PRECLINICAL TO CLINICAL"

As a full-range services partner, we support our global clients from early-stage discovery to market approvals of clients' products.

DEVELOPMENT "FROM CLINICAL TO COMMERCIAL"

As a multicultural high-performing organisation with established strong credibility, we have a stable operational model that deliver optimal level of performance whilst ensuring consistent cGMP manufacturing of safe, cost-effective and reliable products.

COSMETIC PRODUCT SAFETY AND EFFICIENCY

Is your skincare safe? Are your cosmetics doing enough?

When it comes to cosmetics, product safety and efficacy are the two biggest concerns of either legal administration, consumers or manufacturers. Indeed manufacturers have the responsibility of ensuring that each product sold is safe for consumers. It determines brand's credibility and quality.

As consumers expect the product they buy to be efficient and conform to its claims.

Consumers' trust is more and more difficult to get but losing it can be easy when product is unsafe and ineffective. The better the quality perceived, the better it is for brand's image and sales.

COSMECEUTICAL TESTING SERVICES

How to make sure your product is safe? Most of the compulsory tests required in the European Union are already in the cosmeceutical industry. The following are the most common tests to ensure your cosmetic product is safe for consumer use:

1. **Microbiological Testing:** When using a product, customers can bring bacteria that can, mixed with other chemicals, change the product and make it dangerous. This test allows manufacturers to check formulation preservative system and make sure that it is microorganism-free.

Product samples are tested using different methods highlighting the presence of bacteria and/or fungi. The sample is also submitted into a Preservative Effectiveness Test (PET) in order to identify the risk of harmful microorganism's growth.

2. **Stability Testing:** Depending on its environment conditions, a cosmetic product can be altered and become unsafe for consumers. Through stability test, manufacturers are able to ensure that the product maintains its function, physical aspect, as well as its chemical and microbiological quality.

Product samples are put under real conditions to determine its stability and physical integrity- color, odor, texture, etc. It allows manufacturers to evaluate storage conditions and the product shelf life.

3. **Toxicology Testing:** This test is use to determine if any substance/s of the product and their mixtures present a risk when used by customers. Some tests are included to highlight the product effects when in contact with skin and eye.

COSMECEUTICAL TESTING SERVICES

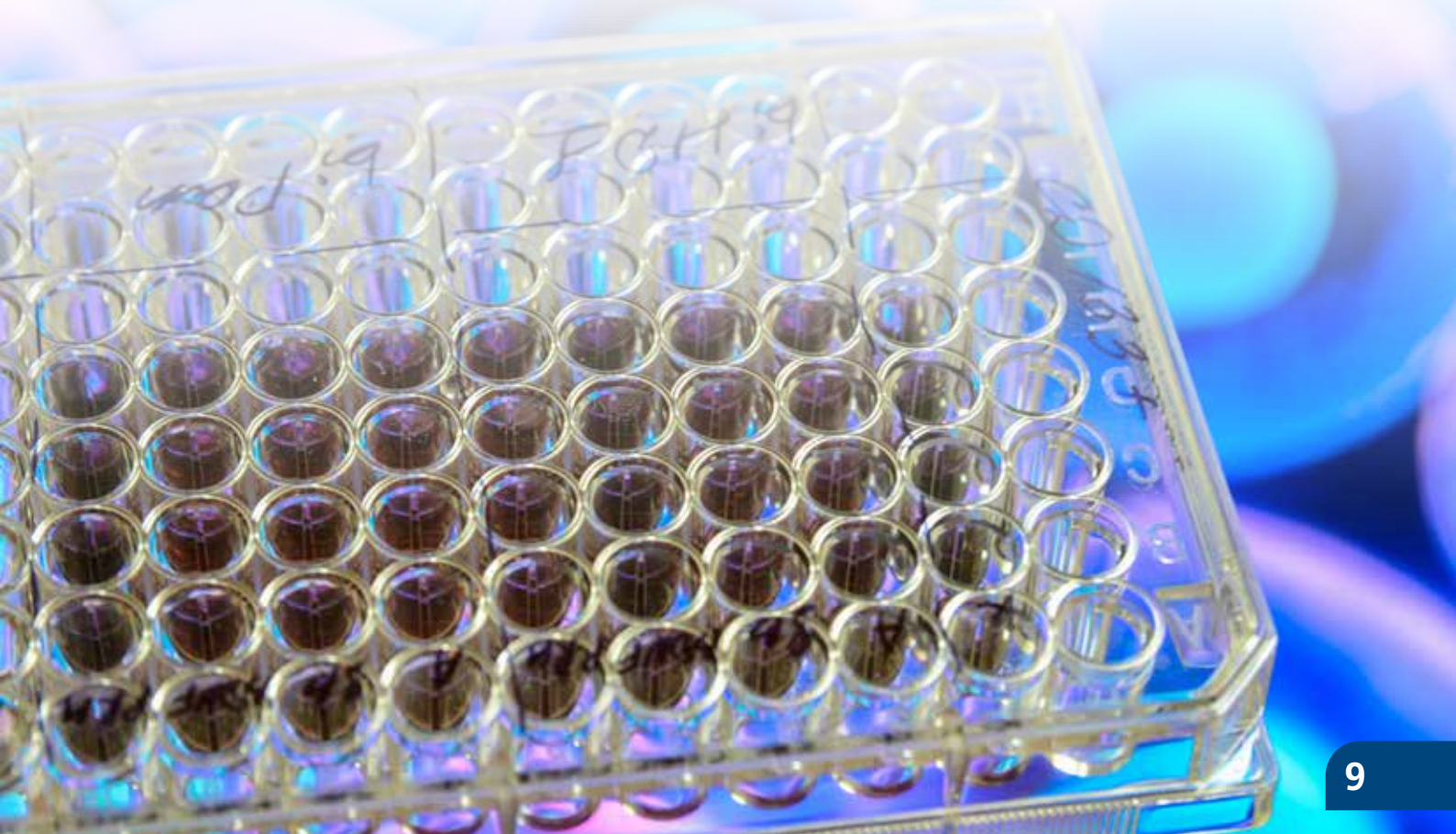
Esco Aster, a CDMO company, offers Total Quality Assurance services and support for new product innovation and development. Our services are also top of the line and will guarantee that all product claims will be backed up by substantial scientific evidences; strengthening consumers' confidence in your specific brands. Our company provides clinical testing methodologies for skin and hair care, specifically:

❑ *IN VITRO* SAFETY STUDIES

Test Category	Test Guidelines
Eye irritation	OECD 437 - *In Vitro* identify Chemicals Inducing Serious Eye Damage
	OECD 492 - In-Vitro Eye Irritation
Skin Irritation	OECD 431 - *In Vitro* Skin Corrosion
	OECD 439 - *In Vitro* Skin Irritation
Skin Absorption	OECD 428 - *In Vitro* Skin Penetration / Absorption (ADS/FDC)
Skin Cytotoxicity	OECD 129 - *In Vitro* Cytotoxicity
Photoxicity Test	OECD 432 - Photoxicity 3T3 NRU
Skin Sensitization	OECD 442C - In Chemico skin sensitization (DPRA/AAPR)
	OECD 442D - *In Vitro* skin sensibility (KerationSens Method)
Genetic Toxicity	OECD 471 - Genetic Toxicity- Ames Test
	OECD 473 - Genetic Toxicity-CA
	OECD 476 - Genetic Toxicity-HPRT
	OECD 487 - Genetic Toxicity-MNT
	OECD 490 - Genetic Toxicity-MLT
Endocrine Disruptor	OECD 455 - ER Transcript Act (Human HeLa9903) Assay
	OECD 456 - H295R Steroidogenesis Assay

In Vitro Identification of Chemicals Inducing Serious Eye Damage -OECD 437	
Title	Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants
Reference	Adapted from Gautheron P. et al (Fundam. Appl. Toxicol. 1992, 18, 442-449) Based on the BCOP protocol from the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) of 2007, the INVITTOX Protocol 124 of 1999 and the information from the Institute for *In Vitro* Sciences (IIVS)
Objective	To identify the corrosives and severe irritants for eye (R41 class)
Test System	Corneas of calves
Methodology	Measurement of the opacity and permeability to fluorescein of calf cornea after contact with the test element under experimental defined according to the organoleptic nature of the product

Eye Irritation using EpiOcular– OECD 492	
Title	Reconstructed human Cornea-like Epithelium (RhCE) Test Method for Eye Irritation
Reference	OECD 492
Objective	Identifying test materials not requiring classification and labelling for eye irritation or serious eye damage
Test system	EpiOcular™ human corneal model
Methodology	Ocular irritation potential is predicted by the relative viability of the tissue after a single exposure to the test substance. Relative viability is determined by measuring the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) dye conversion by the EpiOcular™ tissue construct after topical exposure to the test substance



COSMECEUTICAL TESTING SERVICES

❑ *IN VITRO* SAFETY STUDIES

Skin Corrosion- OECD 431	
Title	Assessment of the Skin Corrosion after Application of a Test Element to Reconstructed Epidermis Model
Reference	Adapted from OECD 431 (updated on July 26, 2013)
Objective	To assess quantitatively the ability of a test element to produce a decrease in cell viability penetrating the stratum corneum by diffusion or erosion after different times of contact
Test system	EPISKIN with stratum corneum from human keratinocytes (3 batches – 1/week)
Methodology	Detection of colour interaction/MTT with the sample, then assessment of the cell viability by staining of the living cells with a vital dye (MTT) and measurement of the mithochondrial activity and reading of the optical densities

Skin irritation – OECD 439	
Title	Assessment of the Irritant Potential of a Test Element on the Reconstituted Epidermis Model
Reference	Approved by the ECVAM (European Centre for the Validation of Alternatives Methods) and by the COLIPA (European Cosmetic Toiletry and Perfumery Industry Association): ATLA, 35, 559-619, 2007 Adapted from OECD 439 (updated on July 26, 2013) Adapted from OECD 439 (updated on July 26, 2013)
Objective	To assess quantitatively the ability of a test element to produce a decrease in cell viability penetrating the stratum corneum after contact for 15 to 60 minutes with the reconstructed epidermis
Test system	Stratum corneum from human keratinocytes (3 units of epidermis)
Methodology	Detection of colour interaction/MTT with the sample, then assessment of the percentage of cell viability by staining of the living cells with a vital dye (MTT) and measurement of the mitochondrial activity and reading of the optical densities

Skin Absorption -OECD 428	
Title	Assessment of the Skin Corrosion after Application of a Test Element to Reconstructed Epidermis Model
Reference	Adapted from OECD 428 (updated on Nov 23, 2004)
Objective	Investigate dermal distribution and absorption of raw materials and finished products
Test system	Franz cells or Bronaugh (dynamic flux) systems or Miniaturized Flow Through Cell (MFTC)
Methodology	This *in vitro* method analysis the permeation and penetration of the test substance. Prior to determination of the dermal absorption of the test substance, the skin integrity is checked. The testing procedure is based on a static or dynamic diffusion cell, using samples of isolated split human skin. the test. The receptor fluid is collected at various time points up to 24 hours. At the end of the experiments, analysis of the various compartments is realised to determine the absorbed amount. A mass balance is also realised to fulfil regulatory requirements.

COSMECEUTICAL TESTING SERVICES

❑ *IN VITRO* SAFETY STUDIES

***In Vitro* Phototoxicity 3T3 NRU – OECD 432**	
Title	Assessment of the Phototoxic Potential of a Soluble Test Element – *In Vitro* 3T3 NRU Photocytotoxicity Test - OECD 432
Reference	Approved by the European commission and its advisory committees (SCCNFP…) and by the ESAC (the ECVAM Scientific advisory committee) on November 3rd, 1997 Validated by the ECVAM (European Centre for the Validation of Alternatives Methods) and by the COLIPA (European Cosmetic Toiletry and Perfumery Industry Association): Toxicology *in vitro*, 12, 305-327, 1998 Application to UV filters: ATLA, 26, 679-708, 1998 Published in the Directive 67/548/EEC relating to the classification of dangerous substances (February 4th, 2000): Method B-41- annex V Adopted by OECD 18 Jun 2019
Objective	To assess quantitatively the phototoxic potential of a soluble test element after exposure to UV
Test system	Balbc 3T3 mouse fibroblasts clone A31
Methodology	Comparison of the cytotoxicity of the test element when tested in the presence and in the absence of exposure (Sol 500 – Dr Hönle) to a non-cytotoxicity dose of simulated solar light. Determination of the cell viability by vital dye uptake (Neutral Red) and appreciation of photo-irritation factor and mean photo effect.

In Vitro Cytotoxicity-OECD 129	
Title	Guidance Document on Using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systematic Toxicity Tests
Reference	Approved and Adapted from OCDE 129 (updated on July 20, 2010)
Objective	To assess the cytotoxic (cell-killing) potential of a test substance
Test System	Cell culture
Methodology	Cell culture prepared in a plate and incubated. Sample will be placed on cultured cell and wash off later. Then cell will be incubated with MTT. Finally, MTT extracted and analyses by spectroscopy.

In Vitro Skin Sensitization– OECD 442D	
Title	Assessment of Skin Sensibility *In Vitro* : KerationSens™ Method
Reference	OECD 442D
Objective	To assess distinction between sensitizing skin products and non- sensitizing skin products to classify and label the dangers of the product in an IATA (Integrated Approaches to Testing and Assessment)
Test system	KerationSens™ cells plate
Methodology	Assessment of Nrf2-ARE activation (Luminescence Reporter Gene Assay) during exposure of keratinocytes to sensitizing products and evaluation of cytotoxicity (MTT)

COSMECEUTICAL TESTING SERVICES

❑ *IN VITRO* SAFETY STUDIES

Genetic Toxicity- OECD 471	
Title	Bacterial Reverse Mutation Test (Ames Test)
Reference	OECD 471
Objective	Bacterial reverse mutation assays use amino acid requiring strains of *Salmonella typhimurium (S. typhimurium)* and *Escherichia coli (E. coli)* to detect point mutations, which involve substitution, addition or deletion of one or a few DNA base pairs
Test system	*5 strains of Salmonella typhimurium (TA 98, TA 100, TA 1535, TA 1537, TA 102) or *4 strains of Salmonella typhimurium (TA 98, TA 100, TA 1535, TA 1537) *1 strain Escherichia coli (WP2 uvrA)
Methodology	According to the direct plate incorporation or the pre-incubation method the bacteria are exposed to the test item with and without metabolic activation and plated on selective medium. After a suitable period of incubation, revertant colonies are counted. At least five different concentrations of the test item are tested with approximately half log (i.e. √10) intervals between test points for an initial test. More narrow spacing between dose levels may be appropriate when a dose response is investigated. For soluble, non-toxic test compounds the recommended maximum test concentration is 5 mg/plate or 5 µL/plate.

In Vitro Genetic Toxicity- OECD 473	
Title	*In Vitro* Mammalian Chromosomal Aberration Test
Reference	OECD 473
Objective	Chromosome aberration assays aim to detect the induction of chromosome breakage (clastogenesis). Although mutagenic substances produce structural chromosome aberrations by a variety of mechanisms, the endpoint is a discontinuity in the chromosomal DNA which is left unrejoined or rejoined inaccurately, thus producing a mutated chromosome.
Test system	Chinese Hamster V79 Cells. Available with human lymphocytes as well.
Methodology	For treatment an asynchronous population of V79 cells in exponential growth should be used. A fixation time of around 20 h after treatment is appropriate since the guidelines recommend fixation times of about 1.5-fold of the normal cell cycle and the normal cell cycle of the used V79 cell line is 12 - 14 h. However, because there may be substances which induce very extensive mitotic delay at clastogenic concentrations or may display their clastogenicity only when cells have passed through more than one cell cycle since the beginning of treatment, an additional later sampling time (28 h) should be included in the second experiment, when indicated. At least three concentrations of the test item with concentration intervals of approximately 2 to 3 fold should be used at fixation time of 20 ± 2 h should be tested. Though the purpose of the assay is to detect structural chromosome aberrations, it is important to report polyploidy and/or endoreduplication when this is seen. Reference mutagens are tested concurrently with the test item in order to demonstrate the sensitivity of the test system. The assay is considered as acceptable, when all three experimental conditions are conducted: short term treatment with and without metabolic activation and long term treatment without metabolic activation.

In Vitro Genetic Toxicity- OECD 476	
Title	*In Vitro* Mammalian Cell Gene Mutation Tests using the Hypoxanthine Phosphoribosyltransferase (HPRT) gene
Reference	OECD 476
Objective	This test is able to assess the potential of the test item to induce gene mutations by means of a HPRT (hypoxanthine-guanine-phosphoribosyl-transferase) assay using the Chinese Hamster V79 cell line. The HPRT system detects base pair mutations, frameshift mutations, small deletions and insertions.
Test system	V79-cells (Chinese hamster cells)
Methodology	HPRT catalyses the conversion of the non-toxic 6-TG (6-thioguanine) to its toxic phosphorylated derivative. In the presence of the HPRT-enzyme, 6-TG is incorporated into cellular nucleotides, resulting in inhibition of cellular metabolism and cytotoxicity. Thus mutant cells are able to proliferate in the presence of 6-TG, whereas normal cells, which contain HPRT, are not. Cells as monolayer cultures are exposed to the test item for a defined period of time (4 h for short time exposure or 20 h for long time exposure). Cytotoxicity is determined by measuring the relative survival (RS) of the cultures. The treated cultures are maintained in growth medium for 7-9 days to allow near-optimal phenotypic expression of induced mutations. Mutant frequency (MF) is determined by seeding defined numbers of cells in medium containing the selective agent (6-TG) to detect mutant cells and in medium without selective medium to determine the cloning efficiency (CE). After a suitable incubation time, cell colonies are counted. The number of mutant colonies in selective medium is adjusted by the number of colonies in non-selective medium to derive the mutant frequency.

COSMECEUTICAL TESTING SERVICES

❑ *IN VITRO* SAFETY STUDIES

***In Vitro* Genetic Toxicity- OECD 490**	
Title	*In Vitro* Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene (Mouse Lymphoma Assay)
Reference	OECD 490
Objective	This *in vitro* experiment is carried out to assess the potential of the test item to induce gene mutations by means of a Thymidine Kinase assay using the mouse lymphoma cell line L5178Y. The Thymidine Kinase (TK) system detects base pair mutations, frameshift mutations, small deletions as well as large, non-lethal deletions and rearrangements of the relevant chromosomes
Test system	L5178Y-cells
Methodology	Cells deficient in the heterozygous TK-locus due to the forward mutation TK +/- ® TK -/- are resistant to the cytotoxic effects of pyrimidine analogues such as TFT. In the presence of TK, TFT is incorporated into the nucleotides, resulting in inhibition of cellular metabolism and cytotoxicity. Thus, mutant cells are able to proliferate in the presence of TFT, whereas normal cells which contain TK, are not. Cells as suspension cultures are exposed to the test item for a defined period of time (4 h for a short-term exposure or 24 h for a long-term exposure). Cytotoxicity is determined by measuring the colony-forming ability and the growth rate of cultures. Mutant frequency is determined by seeding defined numbers of cells in medium containing the selective agent (TFT) to detect mutant cells and in medium without selective agent to determine the cloning efficiency. After a suitable incubation time all colonies are counted. The number of mutant colonies in selective medium is adjusted by the number of colonies in non-selective medium to derive the mutant frequency.

In Vitro methodology of Stably Transfected Transactivation to detect Estrogen– OECD 455	
Title	ER Transcript Act (Human HeLa9903) Assay
Reference	OECD 455
Objective	The aim of this TA assay is to evaluate the ability of a chemical to function as an ERα ligand and activate an agonist response, for screening and prioritisation purposes but can also provide mechanistic information that can be used in a weight of evidence approach.
Test system	hERα-HeLa-9903 stable cell line
Methodology	*In vitro* TA assays are based upon the production of a reporter gene product induced by a chemical, following binding of the chemical to a specific receptor and subsequent downstream transcriptional activation. TA assays using activation of reporter genes are screening assays that have long been used to evaluate the specific gene expression regulated by specific nuclear receptors, such as the estrogen receptors (ERs). Upon reaching 75 – 90% confluency, cells were washed and trypsinised and a single cell suspension is made and seeded into 96-well plates The stock solutions of each reference and test chemical is diluted with 3 µL in 1000 µL serum-free EMEM. Following the incubation period, the test substance in 7 concentrations and the positive and solvent controls are added to the respective wells containing 104 cells and 100 µL 10% FBS - EMEM. The assay plates are incubated for 20 - 24 hours at 37°C and 5% CO2 to induce the reporter gene products. Afterwards, the cell culture supernatant was removed and the cells are further tested according to the protocol of the Luciferase Assay.

In Vitro Chemical effect on steroidogenesis – OECD 456	
Title	H295R Steroidogenesis Assay
Reference	OECD 456
Objective	The Steroidogenesis Assay represents a sensitive method for the analysis of endocrine effects of substances through the production of Testosterone and Estradiol using ELISA. It is possible to differentiate between cytotoxic and endocrine effects of the test item. The Assay does not aim to provide mechanistic information concerning the interaction of the test substance with the endocrine system.
Test system	H295R cells
Methodology	The Testosterone and Estradiol concentration in the cell culture supernatant after 48 hour exposure with the test item is determinate by a competitive ELISA. This enzymatic Read-Out based on the competition between the steroid and a steroid-acetylcholinesterase (AChE) conjugate for a limited amount of steroid antiserum. The antiserum-steroid complex is able to bind to mouse monoclonal anti-rabbit IgG that has been previously attached to the well. After removing any unbound reagents the substrate to AChE is added to the wells. The intensity of the colour after the enzymatic reaction determines spectrophotometrically and is inversely proportional to the amount of free Steroid present in the well. This *in vitro* method analysis the endocrine potential of the test item. The test is carried out using the human adrenocortical carcinoma cell line H295R cultured with different concentrations of the test item. The viability of the cells is tested via the MTT-Viability assay to exclude cytotoxic impacts of the test chemical. The endocrine effect is registered via the Testosterone and Estradiol content of the cell culture supernatants as compared to the solvent control.

COSMECEUTICAL TESTING SERVICES

❑ SKIN EFFICACY TESTING

Cosmetic efficacy tests support and study the real efficacy that packaging or the related advertising messages of each cosmetic product boast. In an increasingly connected and informed world, these have become a fundamental step to inform the consumers about the properties of cosmetics in a simple and scientifically proven way.

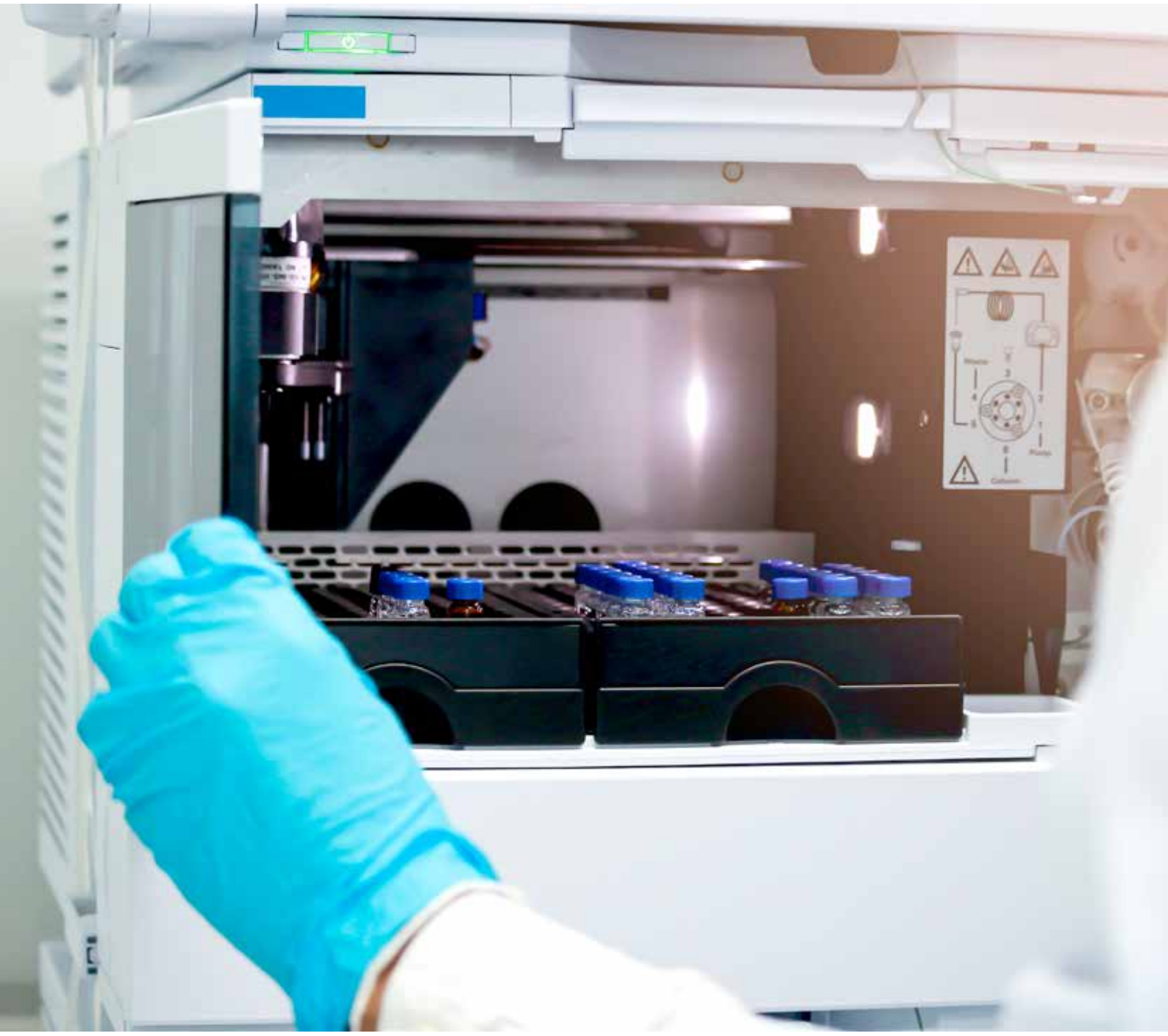
Our laboratories offer a wide range of testing services, thanks to the high competence of the staffs and multiple studies, as well as experts who are able to develop customized proposals for each project.

We apply an integrated multi-instrumental approach that exploits the potential of several techniques to provide a sound evidential support.

COSMECEUTICAL TESTING SERVICES

❑ SKIN EFFICACY TESTING

In Vitro Stratum Corneum Lipid Organization Study- FTIR and DSC	
Title	*In Vitro* Stratum Corneum Lipid Organization Study- Fourier-Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC)
Reference	Internal Method
Objective	To monitor changes in natural lipid content and protein content in the stratum corneum before and after treatment with test substance
Test system	Stratum Corneum
Methodology	Measure Stratum Corneum with FTIR as blank then test substance applied on skin. Test substance wash off from skin and observation again with FTIR to monitoring changes in natural lipid content and protein conformation



Anti-Inflammation	
Title	Anti- Inflammation Test
Reference	Internal Method
Objective	To test if the product triggers inflammation
Test system	3D Human Skin Model, Cell model (keratinocytes, macrophage)
Methodology	• Induce inflammation on the 3D skin model/cell model • Apply test ingredient or formulation and/or controls on the inflamed 3D skin model/cell model • Level of pro-inflammatory cytokines (e.g. IL-6, IL-8) released to be determined by Enzyme-Linked Immunosorbent Assay (ELISA) • Cell viability to be measured by MTT assay • Can be used to justify claims of anti-inflammation.

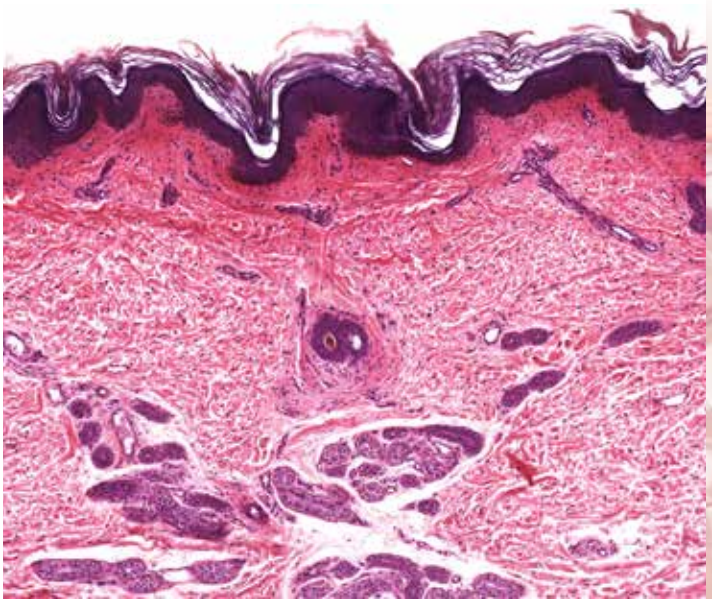
COSMECEUTICAL TESTING SERVICES

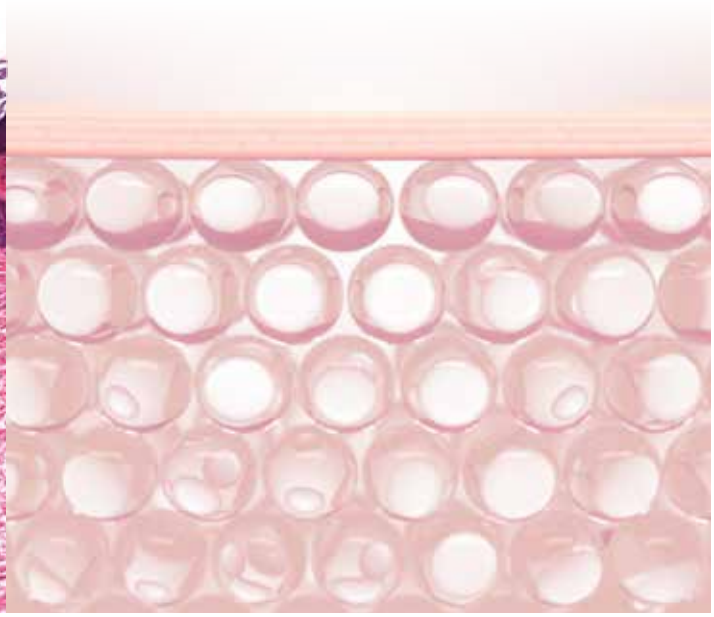
❑ SKIN EFFICACY TESTING

❑ FERMENTATION TECHNOLOGY

***In Vitro* Test for regulation of skin pigmentation**	
Title	*In Vitro* Test for Regulation of Skin Pigmentation
Reference	Internal Method
Objective	To determine whether a product slow down skin pigmentation
Test system	3D Skin models
Methodology	• Apply test ingredient/formulation and positive/negative controls on 3D skin models. • The pigmentation-regulating qualities in the test ingredients, comparative to the controls, can be tracked by: - 14 days photography - Melanin quantification

Title	**Anti-Oxidant Efficacy Test**
Reference	Internal Method
Objective	To test whether the product has anti-oxidant activities
Test system	Human Keratinocytes
Methodology	• Dose the test ingredient on the human keratinocytes • Induce the formation of reactive oxygen species (ROS) in the keratinocytes by exposing to environment stressors • Anti-Oxidant potential of the test ingredient is determined by conducting the DCFH-DA assay





Hydration Skin Test	
Title	Hydration Skin Test
Reference	Internal Method
Objective	To measure transepidermal water loss (TEWL)
Test system	3D Skin Model or human cadaver skin
Methodology	Measure TEWL reading with TEWL probe before and after a test substance was apply on skin model

Histology Skin Observation	
Title	Histology Observation
Reference	Internal Method
Objective	Used to visualize changes in skin layers after exposure to test ingredient or formulation
Test system	3D Skin model, human cadaver skin
Methodology	Test substance apply on human skin or human 3D skin model. After treatment, rinse of the test substance then skin will be fixed, embedded and sectioned, stained and visualize under microscopy.

ESCO
WORLD CLASS. WORLDWIDE

1978

Esco was founded in Singapore and began to pioneer cleanroom technology in Southeast Asia

1990

Esco expands distribution to pharmaceutical life science biotech and medical research markets

2001

Esco earns first EN 12469 cert. for BSC

2006

Esco earns onsite UL accreditation

2007

Expansion of Esco life science and medical products

STRATEGIC ALLIANCES

We adopt an innovative R&D model that encourages external industry and academia collaborations to accelerate open innovation, advance science and technology, and expand market growth opportunities. We collaborate, on a global basis, to complement our CDMO value-added services. Mostly importantly, we encourage the building of new relationships to build new application notes using our tools and technologies.

2017

Esco Aster was introduced (first CDMO company in Asia utilizing its own proprietary Tide Motion technology)

Esco Aster and Instittute of Molecular Cell Biology announce a collaboration to accelerate pre-clinical development of a hand foot and mouth vaccine

Esco Aster and Bioprocessing Institute sign a Memorandum of Understanding to innovate on continuous manufacturing platform

2018

Esco Aster and National University of Singapore announce a development of single-use bioreactor-based stem cells for bone regeneration.

2019

Esco Aster continues to grow with the outfitting of a cGMP-compliant PD, GLP, Phase 1 and 2 clinical trial facility.

Esco VacciXcell was introduced

2015

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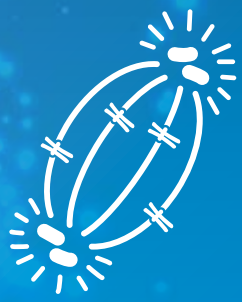

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