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1. COMPANY AND PRODUCT IDENTIFICATION

Product Name: Mitostep (also applies to Mitostep assay kit)

IMMUNOSTEP, S.L.
Avd. Universidad de Coimbra, s/n.

Manufacturer / Supplier: Centro de Investigación del Cáncer
(CIC)

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Information relative to Technical Services: tech@immunostep.com

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Instituto Nacional de Toxicología. Madrid.

2. INFORMATION ABOUT COMPONENTS

2.1. Description:

This product Membrane potential ($\Delta\Psi$) is generated and maintained by concentration gradients of ions such as sodium, potassium, chloride, and hydrogen. Mitochondrial $\Delta\Psi$ drives the accumulation in mitochondria of cationic dyes such as cyanines, and the mitochondrial $\Delta\Psi$ is reduced when energy metabolism is disrupted, notably in apoptosis. Changes in the mitochondrial $\Delta\Psi$ have been described during necrosis, cell cycle and apoptosis. Mitochondrial uptake of dye is a possible source of fluorescence variance. Flow cytometry can be used to estimate membrane potential in eukaryotic cells. Methods using cyanines dyes can detect changes in $\Delta\Psi$. Immunostep MitoStep uses a cationic dye DilCl(5) (1,1',3,3,3'-hexamethylindodicarbo-cyanine idodide) for the study of mitochondrial $\Delta\Psi$. During the apoptosis occurs depolarization of the membrane and as a result there is an increase in cells with less DilCl(5) fluorescence. MitoStep has been optimized for use in flow cytometry, cells stained with DilCl(5) are excited using air-cooled Helium-Neon laser emitting at 633nm, cells DilCl(5) positives emitted at 658 nm. DilCl(5) mean intensity of fluorescence decreases when cells are treated with reagents that induce apoptosis or reagents that disrupt $\Delta\Psi$ mitochondrial.

2.2. Hazardous Ingredients:

COMPONENT Num CAS		EC -No
DilC ₁ (5)	36536-22-8	
DMSO	67-68-5	200-664-3

3. HAZARDS IDENTIFICATION

The toxicity information that follows describes the hazards associated with DMSO. To the best of our knowledge, no other hazards are associated with this product according to Regulation (EC) No. 1272/2008.

- Information pertaining to particular dangers for man and environment associated to DMSO.
- Readily absorbed through the skin. May be irritating to eyes, respiratory system and skin.

Safety datasheet complies with the requirements of Regulation (EC) No. 1907/2006.



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4. FIRST AIDS MEASURES

Information pertaining to DMSO

Description of first aid measures

General advine: Consult a physician. Show this safety data sheet to the doctor in attendance.

If inhaled: If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact: Wash off with soap and plenty of water. Consult a physician.

In case of eye contact: Flush eyes with water as a precaution.

If swallowed: Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water.

Consult a physician.

Most important symptoms and effects, both acute and delayed: Effects due to ingestion may include:, Nausea, Fatigue, Headache

Indication of any immediate medical attention and special treatment needed: Not available

Potencially harmful: Avoid exposure. Wash thoroughly after handling. If eye or skin contact occurs, wash affected area with water for 15 minutes and seek advice. If inhaled, move individual to fresh air and seek medical advice. If swallowed, seek medical advice.

5. FIRE FIGHTING MEASURES

Combustion:	 For small (incipient) fires, use media such as "alcohol" foam, dry chemical, or carbon dioxide. For large fires, apply water from as far as possible. Use very large quantities (flooding) of water applied as a mist or spray; solid streams of water may be ineffective. Cool all affected containers with flooding quantities of water.
Flash Point:	Not determined
Auto Ignition Temperature:	Not determined

6. ACCIDENTAL RELEASE MEASURES

- Wear protective equipment.
- Absorb with liquid-binding material and placed in closed containers for disposal. Avoid generation of aerosols during clean up.
- Ventilate area and wash spill site after material pickup is complete.
- Collect and dispose of all waste in accordance with applicable laws.



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7. HANDLING AND STORAGE

7.1 Handling precautions

- Desiccation recommended
- Store in cool place.
- Store under inert gas. hygroscopic
- Avoid inhaling, ingestion and contact with eyes and skin.
- Avoid exposure to light when not in use

7.2 Storage:

Store at 4°C to 8° C
 No special requirements

 Information about storage in one common storage facility:
 Do not store together with oxidizing and acidic materials as well as

heavy-metal compounds.

Further information about storage conditions:

Keep container tightly closed in a dry and well-ventilated place.

8. EXPOSURE CONTROLS AND PERSONAL PROTECTION

Components with limit values that require monitoring at

the workplace:

The product does not contain any relevant quantities of material with critical values that have to be monitored at th workplace.

Engineering controls: Use in well ventilated area.

Respiratory protection: Not required.

Eye protection: Safety glasses

Body protection: Protective work clothing; impervious gloves, such a latex or equivalent, should be worn to

prevent skin contact

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical state: Liquid

Colour: Blue coloured clear liquid

Odor: Not determined

Change in condition 87 °C - closed cup

Danger of explosion: Not available

Vapour pressure: Not available

Specific gravity 0.42 mm 20° C

Solubility in water: 1.101

pH: High soluble

Melting point/freezing point Melting point/range: 16 - 19 °

Flash point 94°C

Boiling point 189°



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10. STABILITY AND REACTIVITY

Stability:

Material to be avoid
Incompatible material

Acid chlorides, Phosphorus halides, Strong acids, Strong oxidizing agents, Strong reducing agents

Dangerous decompositions products:

Additional information:

11. TOXICOLOGICAL INFORMATION

Carcinogenicity and Toxicity:	None of the components are listed as carcinogens or as suspect carcinogens. Carcinogenicity - rat - Oral Tumorigenic: Equivocal tumorigenic agent by RTECS criteria. Skin and Appendages: Other: Tumors. Carcinogenicity - mouse - Oral Tumorigenic: Equivocal tumorigenic agent by RTECS criteria. Leukaemia Skin and Appendages: Other: Tumors. IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC LD50: 14.5 g/kg, oral, rat; LD50: 8.2 g/kg, intraperitoneal, rat LD50 Oral - rat - 14,500 mg/kg LC50 Inhalation - rat - 4 h - 40250 ppm LD50 Dermal - rabbit - > 5,000 mg/k
Potential effects of chronic exposure:	no data available Genotoxicity in vitro - mouse - lymphocyte Cytogenetic analysis
Germ cell mutagenicity	Genotoxicity in vitro - mouse - lymphocyte Mutation in mammalian somatic cells. Genotoxicity in vivo - rat - Intraperitoneal Cytogenetic analysis Genotoxicity in vivo - mouse - Intraperitoneal DNA damage
Reproductive toxicit	Reproductive toxicity - rat - Intraperitoneal Effects on Fertility: Abortion. Reproductive toxicity - rat - Intraperitoneal Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants). Reproductive toxicity - rat - Subcutaneous Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants). Effects on Fertility: Litter size (e.g., # fetuses per litter; measured before birth).



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Potential health effect	Reproductive toxicity - mouse - Oral Effects on Fertility: Pre-implantation mortality (e.g., reduction in number of implants per female; total number of implants per corpora lutea). Effects on Embryo or Fetus: Fetotoxicity (except death, e.g., stunted fetus). Specific Developmental Abnormalities: Musculoskeletal system. Developmental Toxicity - mouse - Intraperitoneal Effects on Embryo or Fetus: Fetotoxicity (except death, e.g., stunted fetus). Specific Developmental Abnormalities: Musculoskeletal system. Inhalation May be harmful if inhaled. May cause respiratory tract irritation. Ingestion May be harmful if swallowed. Skin May be harmful if absorbed through skin. May cause skin irritation. Eyes May cause eye irritation. Aggravated Medical Condition Avoid contact with DMSO solutions containing toxic materials or materials with
	unknown toxicological properties. Dimethyl sulfoxide is readily absorbed through skin and may carry such materials into the body.
Signs and Symptoms of Exposure	Effects due to ingestion may include:, Nausea, Fatigue, Headache
Additional Information	RTECS: PV6210000

12. ECOLOGICAL INFORMATION

Ecotoxicity:	Contain a component that is harmful to aquatic organisms at high concentrations.
Toxicity to fish	LC50 - Pimephales promelas (fathead minnow) - 34,000 mg/l - 96 h LC50 - Oncorhynchus mykiss (rainbow trout) - 35,000 mg/l - 96 h Toxicity to daphnia and other aquatic invertebrates.
TOXICITY TO HIST	EC50 - Daphnia pulex (Water flea) - 27,500 mg/l Toxicity to algae EC50 - Lepomis macrochirus (Bluegill) - > 400,000 mg/l - 96 h

13. DISPOSAL CONSIDERATIONS.

Waste treatment	Product
methods:	This combustible material may be burned in a chemical incinerator equipped with an afterburner and scrubber. Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.
	Contaminated packaging
	Dispose of as unused product.

14. TRANSPORT INFORMATION

RID /ADR: Non-hazardous for road transport.

IMDG: Non-hazardous for sea transport.

ICAO/IATA: Non-hazardous for air transport.



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15. OTHER INFORMATION

The above information represents the best information currently available for us. However this reagent may present unknown hazards and should be used with caution. Independent professional opinions regarding the risk or exposure to this solution are the responsibility of the user, but does not purport to be all-inclusive and is to be used only as a guide.

This product is intended for use as supplied. Adulteration by dilution or addition of any material to the product as supplied invalidates any diagnostic use of the product.

Immunostep shall not be liable for any damages or losses resulting from the handling of, or from contact with, the product as described in this document.

This product is sold for research purposes only. It is not intended for food, drug, household, agricultural or cosmetic use. The information provided in this safety data sheet is consistent with our knowledge.