

**REPORT METODO ISO 11993-5:2009
BIOLOGICAL EVALUATION “in vitro”:
cytotoxicity test**

**RAPID BRONZ
COD. 013729 LOTTO 259612**

**According the method UNI EN ISO 11993-5 the
biological evaluation “in vitro”: cytotoxicity test.**

RAPID BRONZ

FOREWORD

UNIENISO 10993-5 2009.

This European standard ISO 10993-5 specifies test method and minimum requirements to evaluate the cytotoxicity of chemical substance for different applications in various fields (medical, cosmetic, ecc.).

Under this standard, the test product is tested using in vitro cell culture under defined test conditions, including temperature and contact time, to demonstrate the possible cytotoxic effect and to determine the biological response of mammalian cells in vitro using appropriate biological parameters.

The determination of cytotoxicity depending on the type of evaluation the following:

- a) assessments of cell damage by morphological means;
- b) measurements of cellular damage;
- c) measurements of cell growth;
- d) measurements of specific aspects of cellular metabolism.

IDENTIFICATION OF THE TEST SAMPLE

Description of the sample:

RAPID BRONZ COD. 013729 LOTTO 259612

Cosmetics: emulsion.

Storage conditions: Room temperature.

Date: 07/01/2013

Period of analysis: 2013-01-10 to 2013-02-11.

TEST METHOD A DIRECT CONTACT

The test method of ISO 10993-5 2009 is irritation potential assay of a medical device in vitro on cells derived from skin. The cytotoxic potential of the product to be tested was assessed on cell lines derived from skin: human fibroblasts. Cell viability was assessed by MTT assay. The tetrazolium salt MTT is a yellow color, which reduced by the enzyme succinate dehydrogenase form a precipitate of blue formazan in the mitochondria of viable cells. The precipitate formazan is an indicator of cell viability.

EVALUATION OF CYTOTOXICITY

Cytotoxicity was determined qualitatively and quantitatively:

1) **QUALITY EVALUATION** is to examine the cells under a microscope using a dye cytochemical vital to observe the morphology, the vacuolation, or cell lysis and membrane integrity. The evaluated results were interpreted according to the scale of cytotoxicity following:

0 = Not cytotoxic

1 = Mildly cytotoxic

2 = Moderately cytotoxic

3 = Severely cytotoxic

3 = Not biocompatible: the presence of the irritation and cytotoxic: NOT ACCEPTABLE.

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6.2-RESULTS: TEST MTT Cellule Vero / VERO cells (= fibroblasts)

TABLE 1: Cell viability inhibition (%)

Spettrofotometro: MULTISKAN MS PRIMARY EIA

Concentrazione <i>/concentration mg/ml</i>	% inibizione della vitalità cellulare / <i>Cell viability inhibition (%)</i> Valori medi / <i>Mean</i>	IC ₅₀	RISULTATO / <i>RESULT</i> MTT TEST
RAPID BRONZ COD. 013729 LOTTO 259612			
Controllo positivo/ <i>Positive control: SDS 0.06 mg/ml</i>	0.458 0.408 0.403	< <u>0.5</u>	Citotossico / <i>Cytotoxic</i>
Controllo negativo <i>Negative control:: D-MEM</i>	2.246 2.290 2.200	>1,5	Non Citotossico / <i>Not Cytotoxic</i>
Colture cellulare / <i>cell culture</i>	-	>1,5	Non Citotossico / <i>Not Cytotoxic</i>
C1 1000 µg/ml	2.132 2.208 2.261	>1,5	Non Citotossico / <i>Not Cytotoxic</i>
C2 5000 µg/ml	1.910 1.937 1.887		Non Citotossico / <i>Not Cytotoxic</i>
C3 10.000 µg/ml	1.990 1.962 1.964		Non Citotossico / <i>Not Cytotoxic</i>
C4 20.000 µg/ml	1.820 1.891 1.921		Non Citotossico / <i>Not Cytotoxic</i>
C5 50.000 µg/ml	1.520 1.591 1.521		Non Citotossico / <i>Not Cytotoxic</i>

ESITO:

Il campione in esame RAPID BRONZ COD. 013729 LOTTO 259612 (si presenta NON CITOTOSSICO “in vitro”, in quanto non è stato osservato nessuna percentuale di inibizione cellulare dei fibroblasti Vero e quindi il risultato ottenuto è ACCETTABILE.

6.3-RESULTS: TEST MTT Cellule Balb 3T3 / Balb 3T3 cell

TABLE 1: Cell viability inhibition (%)

Spettrofotometro: MULTISKAN MS PRIMARY EIA

Concentrazione / <i>concentration</i> mg/ml RAPID BRONZ COD. 013729 LOTTO 259612	% inibizione della vitalità cellulare / <i>Cell viability inhibition (%)</i> Valori medi / <i>Mean</i>	IC ₅₀	RISULTATO / <i>RESULT</i> MTT TEST
Controllo positivo/ <i>Positive control</i> : SDS 0.06 mg/ml	0.448 0.410 0.408	< <u>0.5</u>	Citotossico / <i>Cytotoxic</i>
Controllo negativo <i>Negative control</i> :: D-MEM	2.284 2.329 2.248	>1,5	Non Citotossico / <i>Not Cytotoxic</i>
Colture cellulare / <i>cell culture</i>	-	>1,5	Non Citotossico / <i>Not Cytotoxic</i>
C1 1000 µg/ml	1.946 2.105 1.980	>1,5	Non Citotossico / <i>Not Cytotoxic</i>
C2 5000 µg/ml	2.092 1.937 1.887		Non Citotossico / <i>Not Cytotoxic</i>
C3 10.000 µg/ml	1.990 1.962 1.964		Non Citotossico / <i>Not Cytotoxic</i>
C4 20.000 µg/ml	1.964 1.900 1.680		Non Citotossico / <i>Not Cytotoxic</i>
C5 50.000 µg/ml	1.807 1.777 1.729		Non Citotossico / <i>Not Cytotoxic</i>

ESITO:

Il campione in esame RAPID BRONZ COD. 013729 LOTTO 259612 si presenta NON CITOTOSSICO “in vitro”, in quanto non è stato osservato nessuna percentuale di inibizione cellulare dei fibroblasti Vero e quindi il risultato ottenuto è ACCETTABILE.

7- CONCLUSIONS

According to the obtained results, the test product called

RAPID BRONZ COD. 013729 LOTTO 259612

according to UNI EN ISO 10993-5:2009 the product were found be NOT CYTOTOXIC: not presented a irritant effects “in vitro” in cell cultures.