Adamson Analytical Laboratories, Inc.

220 Crouse Dr. Corona, CA 92879 Phone#951-549-9657 Fax#951-549-9659
Analytical Services, Method Development and Formulation Support
Serving Pharmaceutical, Food, and Cosmetics Industries

Contact Person: Jason Koo
Company: Kadesh, Inc.
Address: 9618 Garden Grove Blvd.
Garden Grove, CA 92844
Phone: 714-833-4004
Fax:
Email: jwkoo1004@yahoo.com

Coa Date: 3/21/2016
Date Rec: 2/23/2016

Certificate of Analysis

Sample Name: Puriton (Water) 1500mL
Lot Number: 02222016

AAL No: M1602230001
Sample Description: water, p. bottle BULK
Rush: 0 Rush Terms:
PO: N/A

System Suitability:
Sample RSD:
Equipment Calibration: 5/2015

ANALYSIS	RESULTS	MethNo	Method
Invitro Skin Irritancy	Non-Irritant	EPI-200	invitro
Occular Eye Irritancy	Minimal Irritant (>60minutes)	EPI-200-SIT	Invitro
Invitro Toxicity.	very mild to Non-Irritant	EPI-50-SIT	Invitro

Approved By:

Vicky Seto
Lab Director

Adamson Analytical Laboratories, Inc. sample lot# M16002230001 Occular Report

Run date: 3/17/2016 epioccular OCI-200 3/07/2016 sterile lot#23416 kitA

Ocular Tissue Irritation Summary and Report

The purpose of the Ocular Irritation Protocol for use with the EpiOcular Tissue Model is to determine the relative viability of tissues treated with sample. The relative viability is determined by the ET-50, defined as [time of exposure needed for a test article to reduce the viability of treated tissues to 50% of the control tissues(sic[1])]. Test articles are separated into four categories ranging from severe to minimal irritation. These are:

ET-50 <3min	Severe Irritant	Example:	5% Benzalkonium Chloride
ET-50 3-29.99min	Moderate Irritant	Example:	0.3% Triton X-100
ET-50 30-60min	Mild Irritant	Example:	Pareth 25-12
ET-30 >60min	Minimal Irritant	Example:	Lanolin, Tween 20, diH_2O

For example, if a sample required 45 minutes to reduce tissue viability to 50%, that sample would be classified as a mild irritant. Likewise, a sample that exhibits 30% tissue viability at the 3 minute mark would be classified as a severe irritant.

Tissue viability is determined through a MTT assay. Briefly, ocular tissue cultures are dosed with a fixed amount of sample to varying periods of time and then rinsed to remove any possible irritants to halt the reaction. Tissues are then exposed to MTT for a fixed period of time. Viable tissues convert the MTT to a purple Formazan, which is retained on the cell culture while excess MTT is removed in the subsequent wash. The Formazan is then extracted from the remaining cell culture with 2-Propanol. The sample tissues are compared against the negative control tissue via spectrometry to determine the ET-50.

A humidified incubator at 37.7°C (Spec: 37°C) and 4.3% CO_2 (Spec: 5%) was previously prepared prior to the arrival of the ocular tissue inserts. Upon arrival of the EpiOcular Tissue inserts March 16, the MatTek assay medium was placed into the incubator to warm to 37°C, while the ocular tissue inserts were placed into the refrigerator at 5.6°C. 39 wells in pre-labeled 6-well plates were filled with 900µL pre-warmed assay medium and then the ocular tissue inserts were removed from the refrigerator. The ocular tissue inserts were removed from the assay agar and briefly examined for damage before being placed into an assay medium filled well. The inserts were placed into the incubator at 11:56am and removed at 12:54pm on March 16 2016 (Spec: 1 hour). The assay media in each well was siphoned off, and replaced with 900µL fresh pre-warmed assay medium.

Each insert was dosed with 100µL of test material. Each sample was dosed at three different time periods, 3, 30, and 60 minutes, each done in triplicate. The positive control (PC), 0.3% Triton X-100,

[1] MatTek Corporation, Ocular Irritation Protocol: Neat Method (MTT ET-50)

followed the same procedure as the samples. The negative control (NC), distilled water (molecular biology grade) was dosed with the 60 minute group.

The 60 min exposure group was placed into the incubator at 1:07pm and removed at 2:07pm on March 16,2016 (Spec: 60 min). The 30 min exposure group was placed into the incubator at 1:14pm and removed at 1:43pm March 16,2016 (Spec: 30 min). The 3 min exposure group was placed into the incubator at 1:21pm and removed at 1:24pm on March 16, 2016 (Spec: 3 min).

After removal of each group from the incubator, the inserts were vigorously washed in DPBS to remove test material from the insert, and then placed into a pre-labeled 12 well plate and submerged in 5.0mL pre-warmed assay media. The washed inserts were placed into the incubator at 2:19pm and removed at 2:29pm on March 16, 2016 (Spec: 10 min).

While the inserts were incubating the media wash, the MTT concentrate was added to the MTT diluent to create the MTT solution. 300µL of MTT solution was pipetted into each well of a pre-labeled 24 well plate. The inserts were removed from the media wash and placed into the pre-labeled 24 well plate with MTT solution, ensuring no air bubbles were trapped underneath the cell culture. The inserts were then placed into the incubator at 2:45pm and removed at 5:45pm on March 16 , 2016 (Spec: 3 hours). The inserts were then placed into the refrigerator overnight to cease the MTT/Formazan reaction.

The inserts rinsed with DPBS to remove any excess MTT solution, then blotted with absorbent filter paper and with a kimwipe. The inserts were then placed into a pre-labeled 24 well plate. 2mL of 2-propanol was added to each well containing an insert, and the insert membrane was punctured with a disposable pipette tip to facilitate extraction. The well plates were sealed with parafilm to mitigate evaporation, and then placed onto a slow-shaking vortex at 1:30pm and removed at 3:30pm on March 17th, 2016. The insert was then removed from the Formazan/2-Propanol solution and discarded.

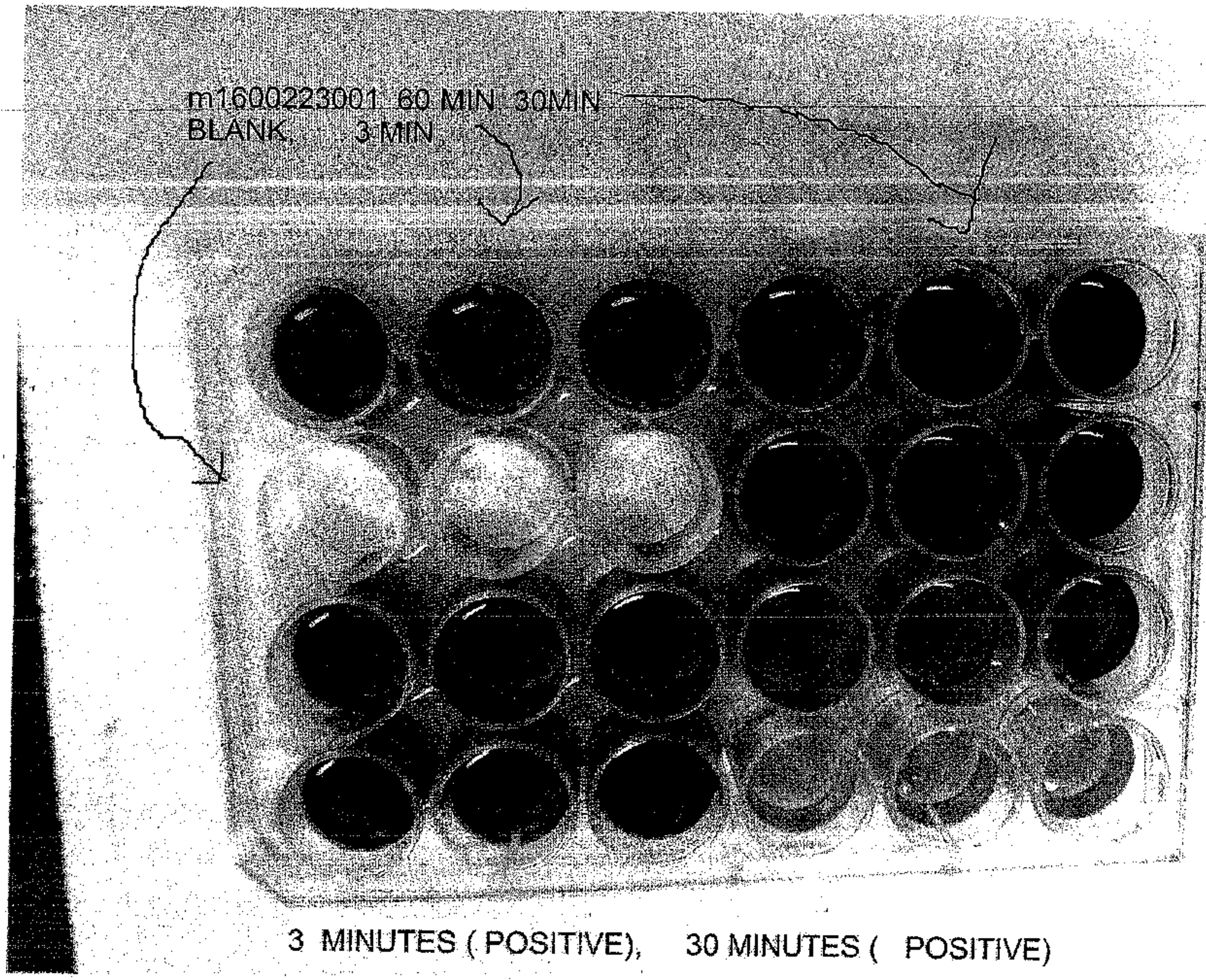
Each solution was thoroughly mixed, then diluted 1:1 with additional 2-Propanol and placed into a cuvette. Each solution was read at 540nm,

Tissue viability was determined by a direct comparison of the absorbance of the NC with each time period of the sample. For these calculations, the ODs at 540nm were used.

Negative Control - distilled water (molecular biology grade)		% Viability	ET-50 (minutes)
60 min exposure	2.809 OD	100%	N/A
Positive Control – 0.3% Triton X-100			37.2
3 min exposure	2.857 OD	101.70%	
30 min exposure	1.885 OD	67.10%	
60 min exposure	0.0.3424 OD	12.17%	
M160223001 P water			>60
3 min exposure	2.901 OD	103.29%	
30 min exposure	2.896 OD	103.12%	
60 min exposure	3.030 OD	107.86%	

The NC and PC match visual expectations. The NC exhibits a deep purple color, indicating excellent cell culture viability via a high Formazan extract. The PC exhibits a Formazan extract that decreases in concentration inversely to dosage time. The calculations confirm 0.3% Triton X-100 as a moderate irritant with a ET-50 37.2 minutes, in accordance to Stern, et al., Toxicology In Vitro, 12, 455-461 (1998).

Samples M1600223001 all have viability values of >60% at 3 minutes, non irritant



3 MIN (SAMPLE)

3 MINUTES (POSITIVE), 30 MINUTES (POSITIVE)

WATER IN CELL(60 MINUTE, POSITIVE CONTROL (60 MINUTES)

KT 03/17/16

EpiOcular™

Part # OCL-200 Ver. 2.0

MatTek Corporation 03/07/2016

Sterile / Lot #23416, Kit A

KT 03/17/16