

**PRODUCT DESCRIPTION**

R&D Systems has developed a set of suspension cell preparations containing different levels of DNA damage to be used as controls with R&D Systems CometAssay® Kits. CometAssay Alkaline Control Cells consist of a healthy population (CC0) and cells treated with increasing doses of Etoposide in populations CC1 (low damage), CC2 (intermediate damage), and CC3 (high damage). When performing alkaline electrophoresis, the four control cell populations show incremental increases in percent DNA in the tail. The healthy control cell population (CC0) was treated with Etoposide under various conditions to increase the amount of damage in populations CC1, CC2 and CC3, respectively. Etoposide is a DNA synthesis inhibitor that induces double-stranded and single-stranded DNA breaks. These cryopreserved controls are designed to act as controls to standardize and compare alkaline electrophoresis methods between individual users and laboratories.

**MATERIALS PROVIDED & STORAGE CONDITIONS**

Do not use past kit expiration date.

PART	PART #	CAP COLOR	AMOUNT PROVIDED	STORAGE OF OPENED MATERIAL
Healthy Control Cells	4256-010-CC0	White	500 µL	CometAssay Alkaline Control Cells are stored using a Liquid Nitrogen Storage System.
Low Damage Control Cells	4256-010-CC1	Yellow	500 µL	
Intermediate Damage Control Cells	4256-010-CC2	Green	500 µL	
High Damage Control Cells	4256-010-CC3	Red	500 µL	

**OTHER MATERIALS REQUIRED**

**Reagents:**

- Ice cold 1X PBS pH 7.4, Ca<sup>++</sup> and Mg<sup>++</sup> free (R&D Systems, Catalog # 4870-500)
- Isopropanol

**Equipment:**

- Pipettes and pipette tips
- Table Top Centrifuge (vertical rotor)
- Water Bath
- ≤ -70°C Freezer
- Liquid Nitrogen Storage System

**PRECAUTION**

When handling bio-hazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

**LIMITATIONS**

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- Results may vary due to variations among tissue/cells derived from different donors or sources.

## PREPARATION OF CONTROL CELLS

Control cells should be prepared immediately before starting the CometAssay protocol.

### STORAGE

CometAssay Control Cells are stored using a Liquid Nitrogen Storage System. To avoid the accumulation of damage due to freeze thaw, the control cells should be aliquotted and cryopreserved as described below.

1. Recover cells by submerging in 37 °C water bath to quickly thaw cells, and place on ice.
2. Gently invert to mix and transfer 50 µL aliquots into freezing vials.
3. Freeze at  $\leq -70$  °C with  $-1$  °C per minute freezing rate. This can be done by placing the vials in a Styrofoam box containing room temperature Isopropanol and placing in the freezer overnight. Vials are placed in a plastic box or rack then placed in room temperature isopropanol. The lid of the Styrofoam container is put in place then the box is placed in the  $\leq -70$  °C freezer.
4. Transfer to Liquid Nitrogen System for long-term storage.

### ASSAY PREPARATION PROTOCOL

1. Remove 50 µL aliquots of CC0, CC1, CC2 and CC3 CometAssay Alkaline Control Cells from Liquid Nitrogen Storage.
2. Quickly thaw cells by submerging in 37 °C water bath, and add 600 µL of ice cold 1X PBS (Ca<sup>++</sup> and Mg<sup>++</sup> free).
3. Centrifuge cells at 150 x g for 5 minutes and gently remove supernatant, except for about 50 µL.

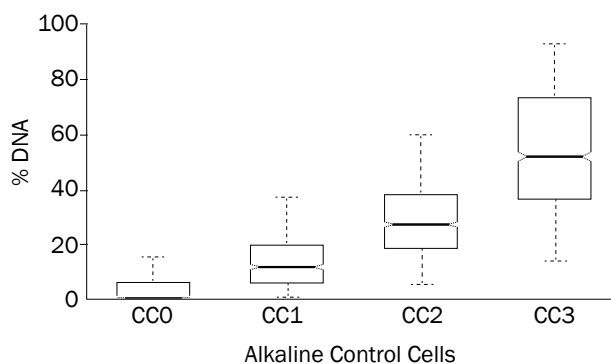
**Note:** 1) A cell pellet will not be visible after centrifugation.

2) Removing supernatant completely will result in cell loss.

4. Gently resuspend cell pellet in 50 µL of ice cold 1X PBS.
5. Immediately use the cells in the CometAssay protocol described for Alkaline CometAssay.

### EXAMPLE DATA

These cell preparations containing different levels of DNA damage are to be used as controls with CometAssay Kits. Lot specific data is generated to calculate percent DNA in tail for each control cell population (CC0-CC4).



% DNA BY ALKALINE CONTROL CELLS	N	MEAN	SD	SE	80% CI	MEDIAN	IQR
CC0	378	3.735	5.414	0.2785	0.420-0.550	0.440	6.278
CC1	377	14.105	11.953	0.6156	10.650-12.530	11.590	13.843
CC2	372	29.343	16.467	0.8538	26.160-28.810	29.385	19.114
CC3	455	53.573	24.006	1.1254	49.070-54.180	51.700	36.967

**Figure 1: Damage in CometAssay Alkaline Control Cells Detected as Percent DNA in Comet Tails.** Example of the data obtained is expressed as a Box-Whisker plot and it is specific to each lot of cells manufactured. Table shows raw data for Box-Whisker plot.