

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 Neutral Control Cells consist of a healthy population (NC0) and cells treated with increasing doses of Bleomycin in populations CC1 (low damage), NC2 (intermediate damage), and NC3 (high damage). When performing the neutral CometAssay, the four control cell populations show incremental increases in tail moment. The healthy control cell population (NC0) was treated with Bleomycin under various conditions to increase the amount of damage in populations NC1, NC2 and NC3, respectively. Bleomycin complexed with metal ions induces double-stranded and single-stranded DNA breaks by a mechanism involving free radicals. These cryopreserved controls are designed to act as controls to standardize and compare comet assay results under neutral electrophoresis conditions 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	4257-010-NC0	White	500 µL	CometAssay Neutral Control Cells are stored using a Liquid Nitrogen Storage System.
Low Damage Control Cells	4257-010-NC1	Yellow	500 µL	
Intermediate Damage Control Cells	4257-010-NC2	Blue	500 µL	
High Damage Control Cells	4257-010-NC3	Green	500 µL	

OTHER MATERIALS REQUIRED

Reagents:

- Ice cold 1X PBS pH 7.4, Ca⁺⁺ and Mg⁺⁺ 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

Neutral 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.

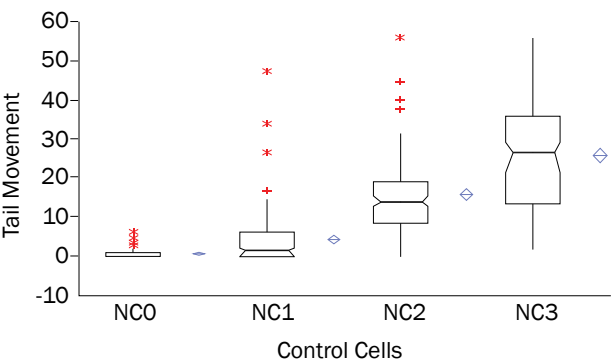
- 1. Recover cells (5 x 10⁵ cells/mL) by submerging in 37 °C water bath to quickly thaw cells, and place on ice.
- 2. Gently invert to mix and transfer 50 µL aliquots into freezing vials.
- 3. Freeze at ≤ -80 °C with -1 °C per minute freezing rate. This can be done by placing the vials in a Styrofoam box containing room temperature Isopropanol and placing in a ≤ -80 °C 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 ≤ -80 °C freezer.
- 4. Transfer to Liquid Nitrogen System for long-term storage.

ASSAY PREPARATION PROTOCOL

- 1. Remove 50 µL aliquots of NC0, NC1, NC2 and NC3 CometAssay Neutral Control Cells (~5 x 10⁵ cells/mL) from Liquid Nitrogen Storage.
- 2. Quickly thaw cells by submerging in 37 °C water bath and add 500 µL of ice cold 1X PBS (Ca⁺⁺ and Mg⁺⁺ free).
- 3. Centrifuge cells at 300 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 suspend cell pellet in ice cold 1X PBS to ~1-2 x 10⁵ cells/mL.
- 5. Immediately use the cells in the CometAssay protocol described for Neutral 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 (NC0-NC3).



TM By Bleomycin	N	MEAN	SD	SE	75% CI OF MEAN	MEDIAN	IQR	75% CI OF MEDIAN
NC0	75	0.677	1.2410	0.1433	0.511-0.843	0.000	0.637	0.000-0.140
NC1	75	4.316	7.7817	0.8986	3.274-5.358	1.360	5.748	0.240-2.510
NC2	75	15.711	10.7829	1.2451	14.268-17.155	1.600	10.117	12.830-14.950
NC3	75	25.370	13.7918	1.5925	23.884-27.577	26.780	22.750	20.810-28.930

Figure 1: Box-Whisker plot of Neutral CometAssay Control Cells: Tail Moment. 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.