



Monitoring DNA synthesis rate in A431 cells after external irradiation

APPLICATION NOTE

Proliferation status of irradiated cells can be monitored by use of ^{14}C -labeled thymidine. The higher the irradiated dose, the more DNA damage and consequently the longer time is required for the cells to recover. This effect can be observed through ^{14}C -thymidine incorporation in DNA. This application note describes how the ^{14}C -thymidine incorporation rate depends on the irradiated dose as monitored using LigandTracer[®] White.

Experimental procedures

The human squamous carcinoma cell line A431 (CLR 1555, ATCC, Rockville, MD, USA) was seeded evenly on a whole petri dish surface (dish size 100x20) and left to grow to confluency (50 to 60%). LigandTracer White was placed in an incubator (5% CO_2 , 37°C) well before the measurements started so that the instrument reached thermal equilibrium.

Cells were irradiated with increasing dosage level (3, 6, 10 and 30 Gy, for each level a different petri dish). As indicated in Figure 1, a small area of cells was lead-shielded (A) and another one was cleared from cells (B) with a cell-scraper. The cell dish was placed in LigandTracer White and ^{14}C -thymidine (~46 kBq) was added. The speed of incorporation of ^{14}C -thymidine in shielded cells and irradiated cells were measured simultaneous for about 7 hours.

Results

External irradiation of A431 with 6 Gy or more clearly slows down the DNA synthesis process. Figure 2 shows the ^{14}C -thymidine signal from irradiated cells and protected cells measured continuously after irradiation with 10 Gy. Figure 3 shows noise-reduced curves from the different doses. The effect of 30 Gy and 10 Gy is similar, 6 Gy slows down the DNA synthesis to some extent, and 3 Gy did not impact the DNA synthesis rate.

Conclusions

Cell proliferation as monitored using ^{14}C -thymidine can be measured in real-time using LigandTracer White. The data can be used for the quantification of how external irradiation affects the proliferation speed. Other applications include proliferation measurements following exposure to cytotoxic drugs or replication inhibitors and onset of S-phase after G1 synchronization.

References

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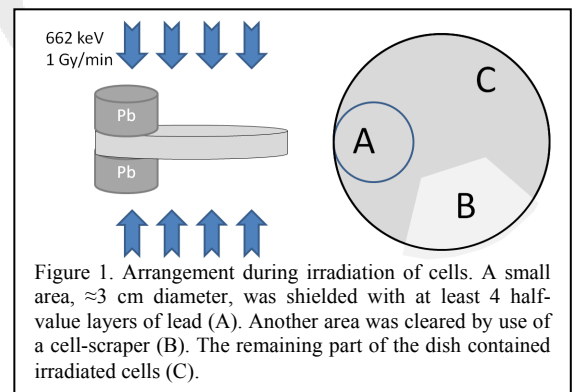


Figure 1. Arrangement during irradiation of cells. A small area, ≈3 cm diameter, was shielded with at least 4 half-value layers of lead (A). Another area was cleared by use of a cell-scraper (B). The remaining part of the dish contained irradiated cells (C).

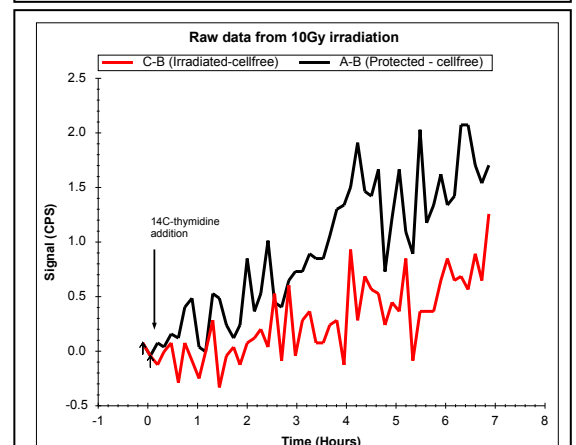


Figure 2. DNA synthesis rate of A431 cells after irradiation with 10 Gy.

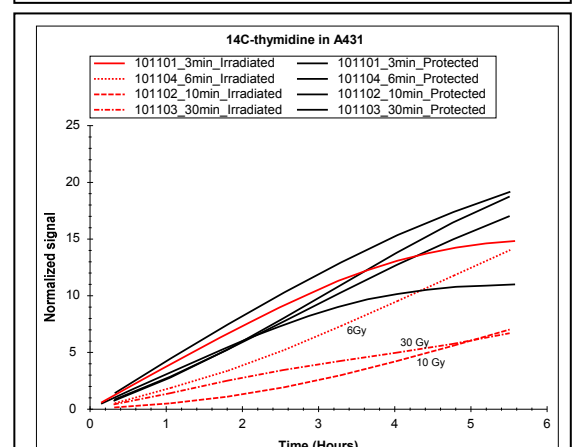


Figure 3. DNA synthesis rate from four different irradiation Trend-lines from protected areas and irradiated areas