

# Uncover apoptosis: a sensitive and sensible approach

## Invitrogen BioSource™ phosphoELISA™ assays for studying apoptosis

Apoptosis, or programmed cell death, is essential to the development, immunological competence, and homeostasis of living things. It has become one of the most widely researched cell processes in biology, with over 1,200 articles published monthly in the past year alone.

Induction of apoptosis unfolds a cascade of events that triggers the activation of effector caspase proteases. Caspase proteases then cleave poly (ADP-ribose) polymerase (PARP), a 116 kDa nuclear protein typically involved in DNA damage detection and repair, between Asp214 and Gly215. This cleavage produces the p85 and p25 fragments, effectively eliminating DNA repair by PARP during apoptosis and committing the cell to the apoptotic pathway (Figure 1).

Many assays designed for examining apoptosis rely upon the activation of caspases. However, caspase proteases are rapidly degraded, making them difficult to detect and often overlooked by existing methods. Fortunately, there are other targets available for measuring apoptotic activity. Since PARP cleavage plays a significant role in apoptosis, PARP, p25, and p85 are ideal markers for these assays.

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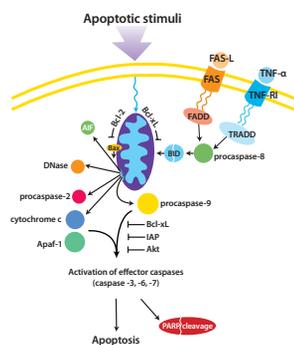


Figure 1—Pathway of PARP cleavage by caspases.

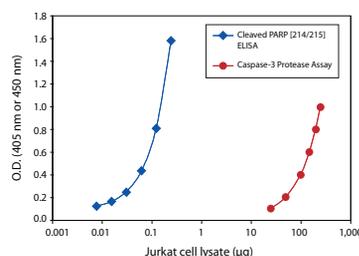


Figure 2—Sensitivity comparison of the Cleaved PARP [214/215] phosphoELISA™ Kit to a caspase-3 protease assay. Jurkat cells were treated with 1 µM staurosporine for 3 hours. Cell extracts were prepared. Cell lysates were serially diluted and analyzed with the Cleaved PARP [214/215] phosphoELISA™ Kit (Cat. no. KHO0741) and Caspase-3 Colorimetric Kit (Cat. no. KHZ0021). The amount of cell lysate assayed was plotted against the corresponding O.D. signal.

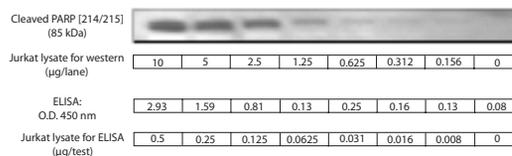


Figure 3—Detection of cleaved PARP [214/215] by ELISA and western blot. Jurkat cells were treated with staurosporine. The amounts of cell lysate used in western blotting and ELISA are indicated. Different amounts of cell lysate were used due to the much higher sensitivity of ELISA. The bands shown in the western blotting data were developed using rabbit anti-cleaved PARP [214/215] (Cat. no. 44-698G) and an alkaline phosphatase-conjugated anti-rabbit IgG followed by chemiluminescent substrate and autoradiography.

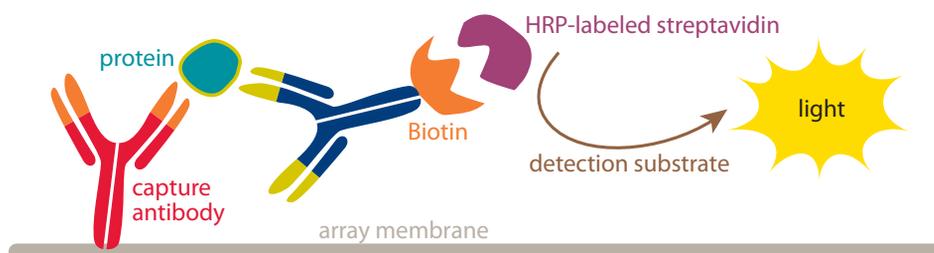


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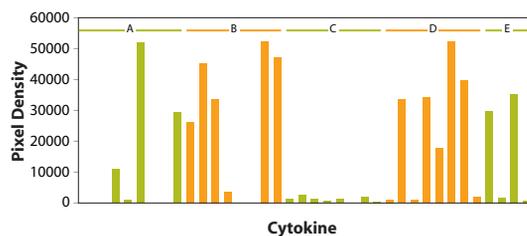
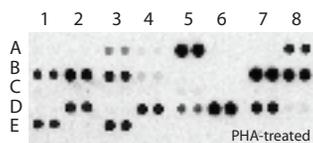
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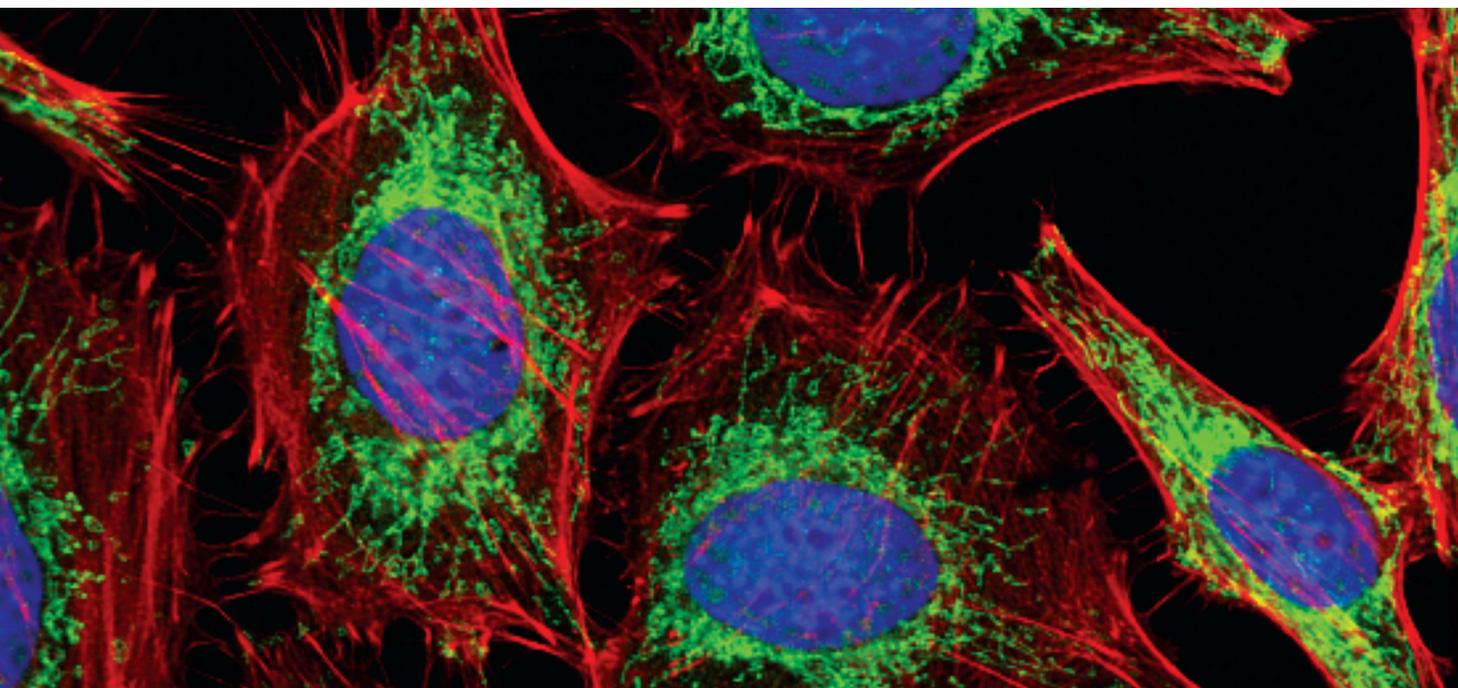


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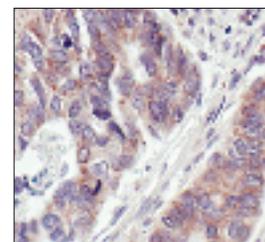
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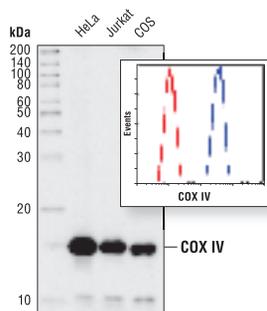
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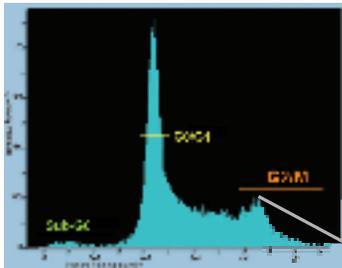
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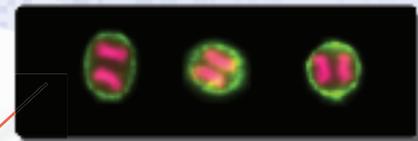
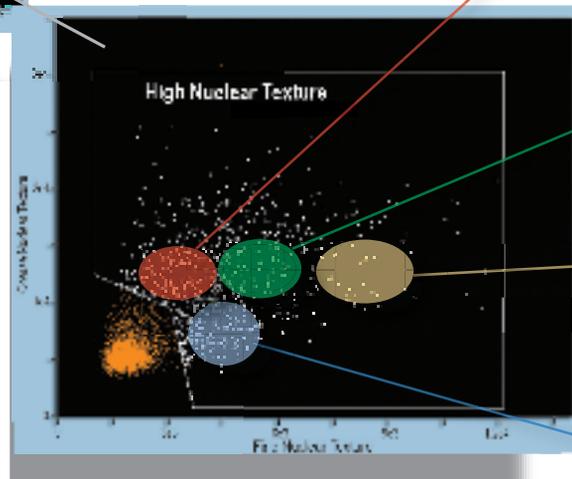
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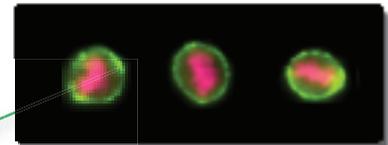
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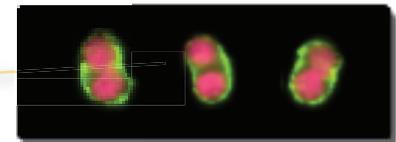
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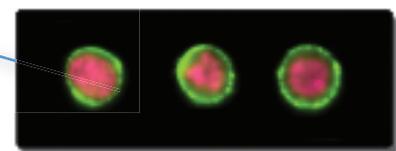
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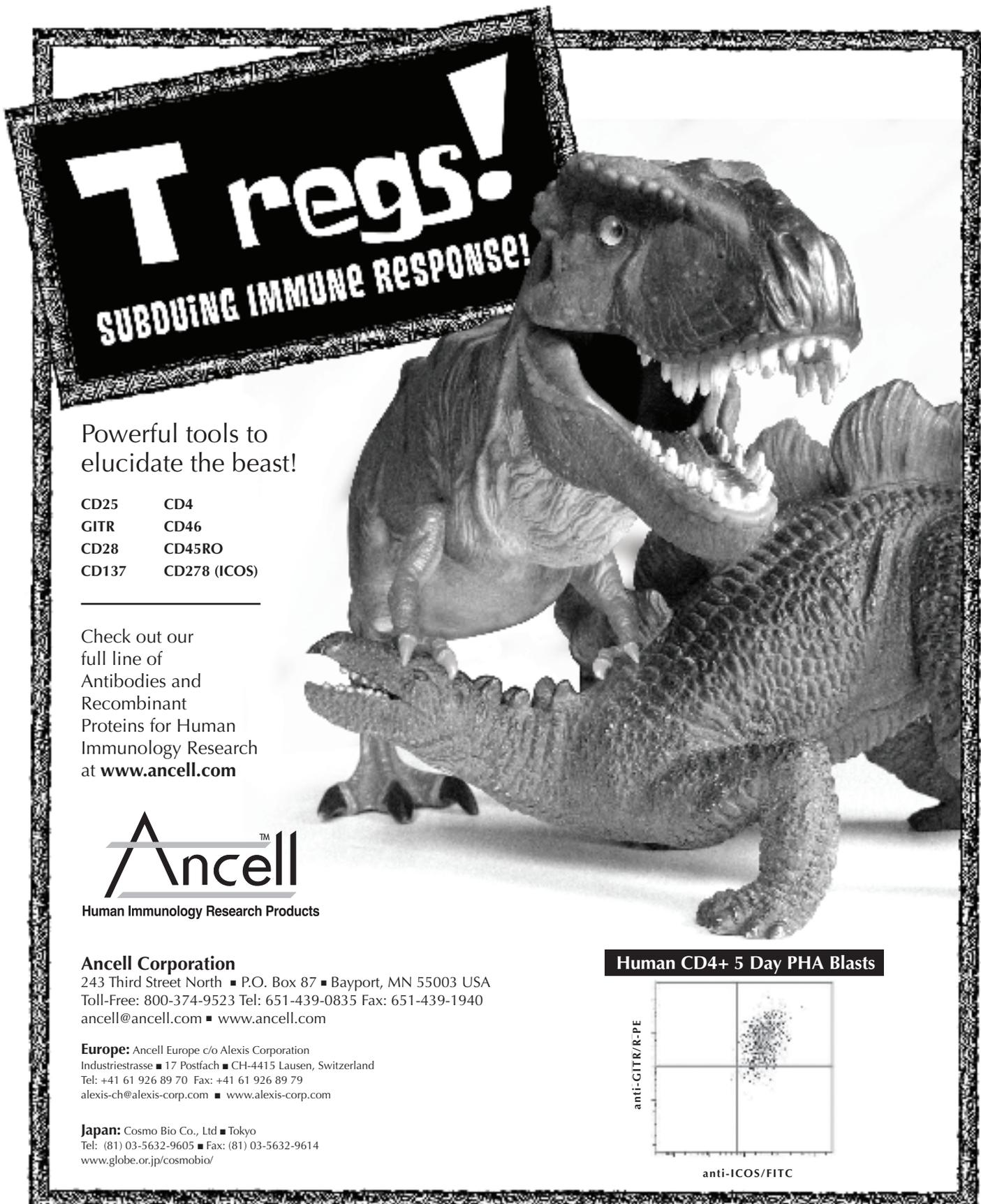


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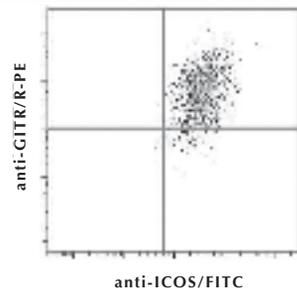
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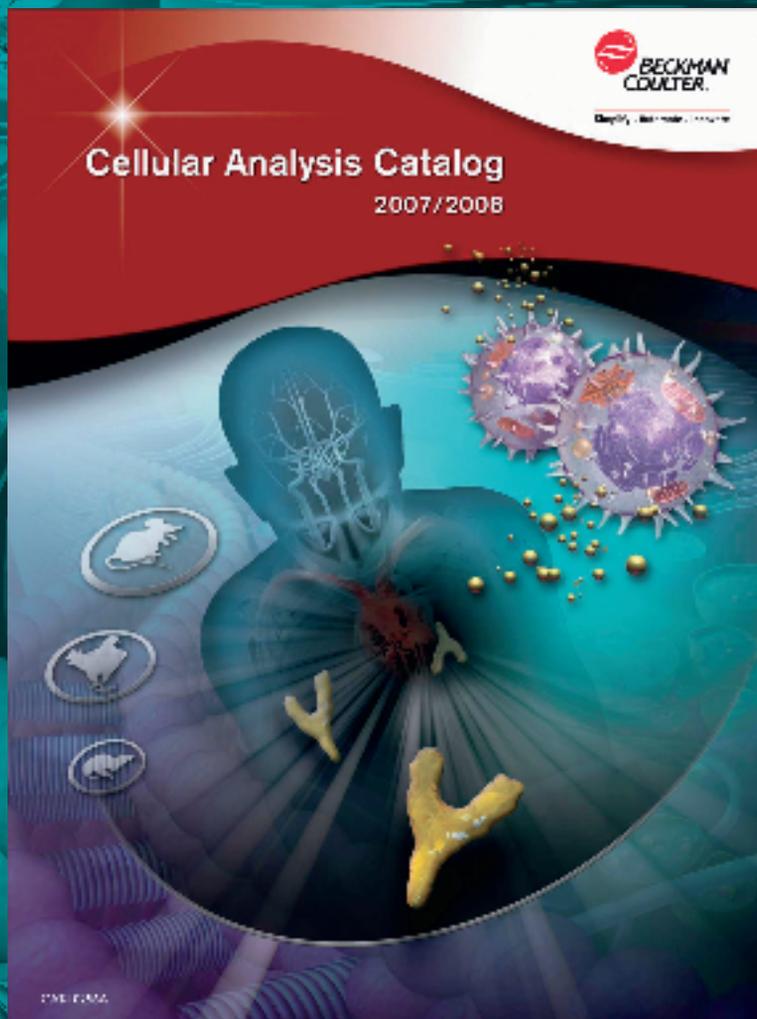
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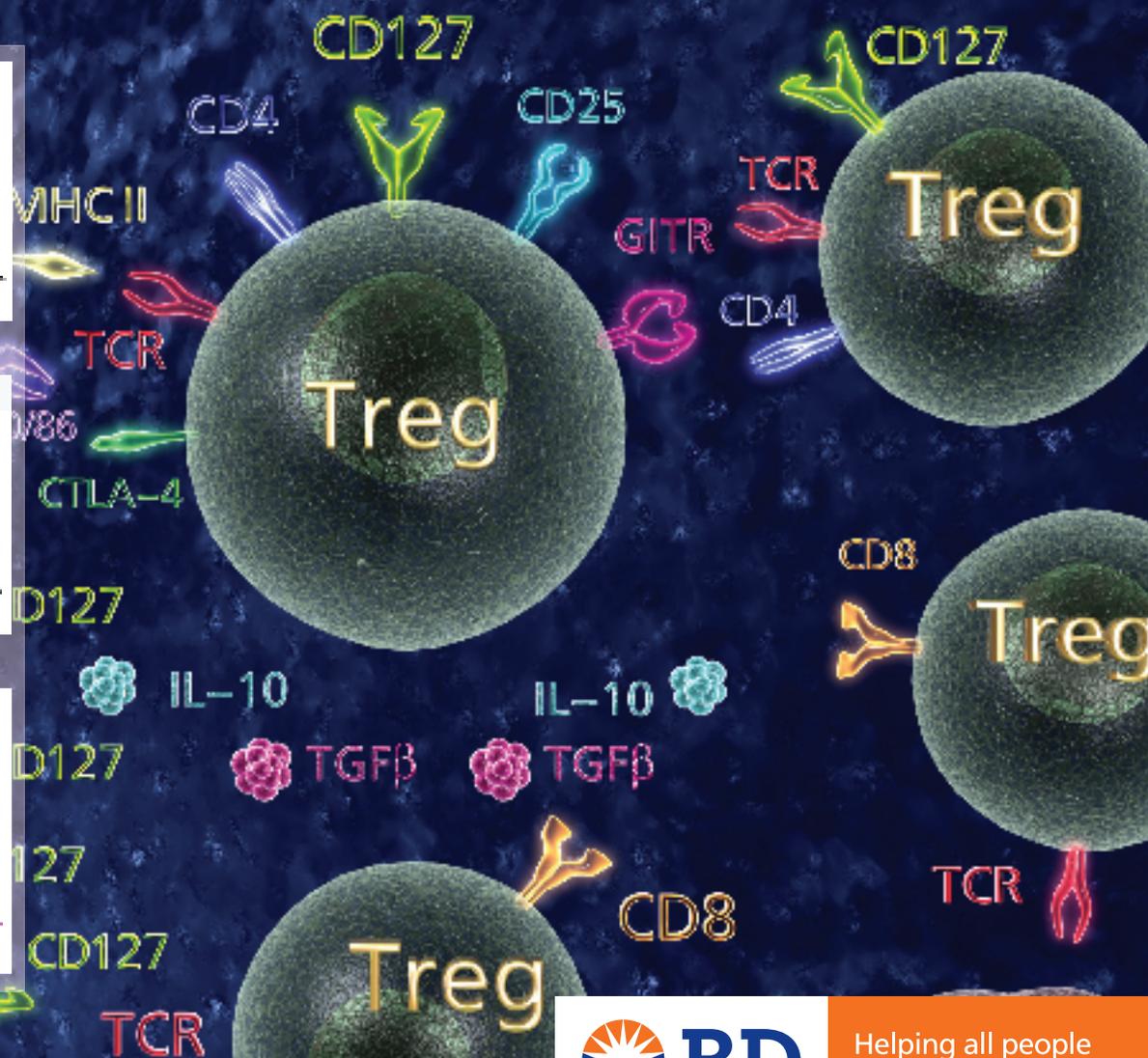
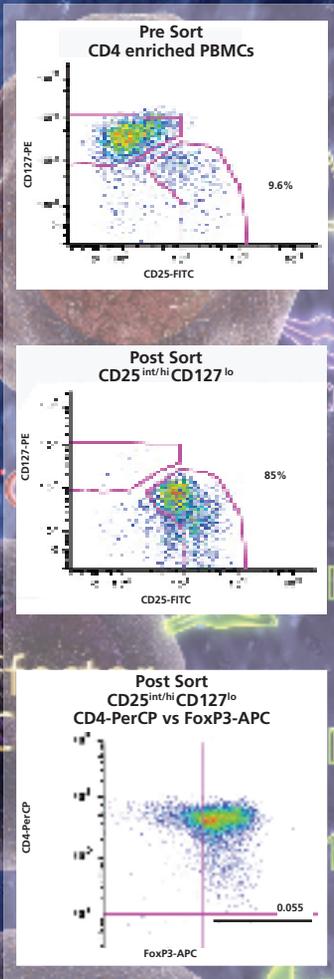
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