# Viahance™: Material Data Sheet

## The Product


Viahance is provided as a 2 ml or a 10 ml fill, which can purify approximately 20 or 100 cell samples, respectively. Separation can be achieved using a simple button-magnet source. Therefore, expensive magnetic filters are not required.

## General Information

Viahance dead-cell removal kit is used to enhance the live to dead cell ratio in cell culture through removal of dead cells and cellular debris using magnetic negative selection. Viahance removes dead cells, stripped nuclei and free oligonucleotides. Competing dead cell separation kits only remove dead cells.

Viahance's ability to remove dead cells, stripped nuclei, and free oligonucleotides from your cell culture will increase cell culture purity, improve data quality and favorably impact downstream results. Eliminating stripped nuclei and free oligonucleotides cleans up a cell preparation for future molecular biology work. As important, Viahance does not contain foreign proteins (such as Annexin-V) and is provided autoclaved.

## Demonstration of Performance – Timing of Magnetic Separation

The timing of magnetic separation is an important parameter to consider when using a simple button magnet for dead cell removal. When magnetic separation is too short, all the dead cells are not removed. When magnetic separation is too long, the live cells begin to settle to the bottom of the tube lowering the recovery of live cells. Figure 1 illustrates the effect of magnetic separation time on yield of live cells in the presence of Viahance. The yield of live cells in the supernatant falls quickly due to simple gravimetric removal of the live cells from the supernatant. Based on this study, an upper limit of 15 minutes for magnetic collection is recommended.

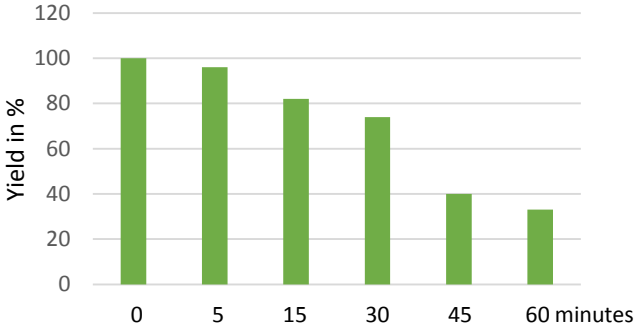


**Figure 1**: Effect of magnetic separation time on the recovery of NIH-3T3 live cells using 350 µg Fe of Viahance. Cells were treated with Viahance for 5 min followed by the indicated magnetic separation times.

Similarly, the magnetic separation time on dead cell capture and removal using Viahance is also an importance parameter. Figure 2 shows that the percent of dead cells in the supernatant falls quickly after magnetic removal from the supernatant at times as short as 5min but is minimally changed with longer separation times. These optimization studies were performed using NIH-3T3 cells and a simple magnetic button source separator. Researchers should define these parameters for their unique applications.

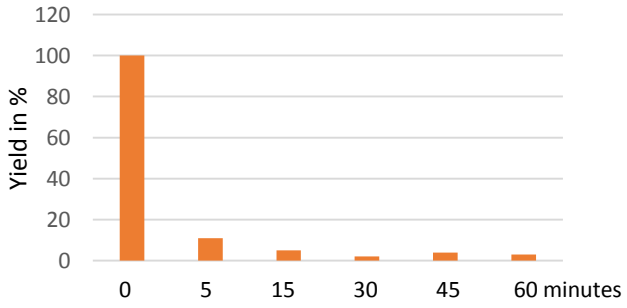


**Figure 2**: Effect of magnetic separation time on the removal of NIH-3T3 dead cells using 350 µg Fe of Viahance . Cells were treated with Viahance for 5 min followed by the indicated magnetic separation times.

# Demonstration of Performance – Viahance Concentration

Non-specific binding of Viahance to live cells is another important parameter to consider. Non-specific binding of Viahance to live cells could limit the availability of Viahance for binding to dead cells while simultaneously removing live cells during magnetic separation. To investigate this parameter a fixed number of live NIH-3T3 cells (500,000) were treated with increasing dosages of Viahance. Recovery of live NIH-3T3 cells was unaffected by Viahance (Figure 3).

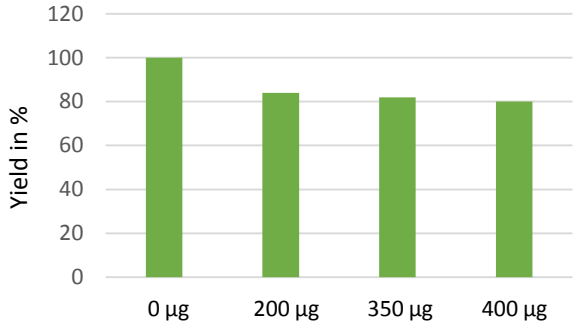


**Figure 3**: Yield of live NIH-3T3 cells treated with increasing amounts of Viahance using a 5 min initial incubation followed by a 15 min separation time (the Standard Protocol).

The amount of Viahance used to separate dead cells from live cells is another important parameter to consider when designing a live-dead cell purification protocol. Figure 4 shows that the percent of NIH-3T3 dead cells in the supernatant falls quickly after a 15 minute magnetic separation using 50 µg Fe Viahance. The completeness of dead cell removal increases slowly out to 400 µg Fe Viahance. BioPAL recommends using a 200 µg Fe of Viahance for separation of 500,000 to 10,000,000 cells. Researchers should define this parameter for their unique applications.

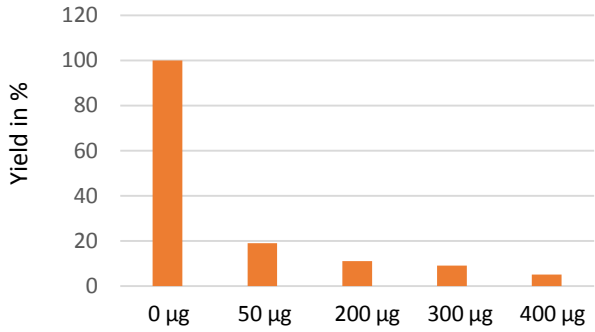


**Figure 4:** Yield of NIH-3T3 dead cells treated with increasing amounts of Viahance using the Standard Protocol.

# Demonstration of Performance – DNA Removal

The effect of increasing amounts of Viahance on a fixed amount of DNA was determined by adding 40 to 800 µg of Viahance to 1ml of DNA (130 µg/ml). After magnetic separation, the optical density (A400) of the magnetically separated supernatant was determined (Table 1). The results show that 130 µg of DNA quantitatively removes 200 µg of Viahance.

| | Plus DNA | Minus DNA |
|---|---|---|
| Viahance (µl) | Readout at A400 | |
| 40 | 0 | 0.4 |
| 80 | 0 | 0.8 |
| 200 | 0 | >3 |
| 400 | 2.2 | >10 |
| 800 | >10 | >10 |

**Table 1:** Binding of a variable amount of Viahance to a fixed amount of DNA.

The effect of increasing amounts of DNA on a fixed amount of Viahance was determined by adding 40 to 320 µg of DNA to 50 µg of Viahance. After magnetic separation, the optical density (A260 ) of the magnetically separated supernatant was determined (Table 2). The results show that 50 µg of Viahance quantitatively removed 160 µg of DNA.

| | Plus Viahance | Minus Viahance |
|---|---|---|
| DNA (µg) | Readout at A260 | |
| 0 | 0 | 0 |
| 40 | 0 | 0.66 |
| 80 | 0 | 1.4 |
| 160 | 0 | 2.8 |
| 320 | 2.3 | 5.6 |

**Table 2:** Binding of a variable amount of DNA to a fixed amount of Viahance.

# Comparison of DNA Removal – Viahance and MACS Annexin V

An important advantage of Viahance is its ability to simultaneously remove dead cell and nucleic acid material from cell culture. Competing products such as MACS® Annexin V leave nucleic acids behind.

Equal volumes (200 µl) of either Viahance or MACS® Annexin-V were added to DNA (~70 µg) dissolved in 1ml of PBS. The solutions were gently mixed and incubated for 5 minutes. After incubation, the solutions were placed on a magnetic separator for 30 minutes and then the optical density (A260) of the magnetically separated supernatants was determined (Table 3). In parallel, the experiment was repeated without the addition of DNA. The results show that Viahance binds DNA forming an aggregate and the aggregate is magnetically removed whereas MACS® Annexin-V does not interact with DNA and the DNA is not magnetically removed.

| Viahance | PBS | PBS + 65 µg DNA | | MACS® Annexin V | PBS | PBS + 65 µg DNA |
|---|---|---|---|---|---|---|
| | Readout at A260 | | | | Readout at A260 | |
| 0 µl | 0.00 | 0.88 | | 0 µl | 0.00 | 0.91 |
| 200 µl | 2.18 | 0.04 | | 200 µl | 2.25 | 2.61 |

**Table 3:** Comparison of DNA removal by Viahance and MACS® Annexin-V.

# Comparison to Competing Products

| | Viahance | MACS® Annexin V | PromoKine Annexin V |
|---|---|---|---|
| Reagent preparation | Ready to use | Ready to use | Multiple washes |
| Basis of separation | NP binding to nuclear DNA followed by magnetic separation | NP binding to membrane associated phosphatidyl serine by Annexin V followed by magnetic separation | Binding to phosphatidyl serine by an Annexin V biotin conjugate followed by binding of complex by a Streptavidin micromagnetic bead followed by magnetic separation |
| Assessment of assay procedure | Simple | Medium | Complex |
| Steps to complete | 2 | 2 | 15 |
| Time to complete, min | 20 | 30-40 | 300 |
| Temperature of separation | RT | RT | 4°C and RT |
| Maximum cell removal per procedure | ~$10^8$ | 2 x $10^8$ | $10^7$ |
| Magnetic separation | Simple magnet button separator | Magnetic filter plus 360° magnetic separator | Simple magnet button separator |

**Table 4:** Comparison of Kit Methods.

| | Viahance (-) autoclaving | Viahance (+) autoclaving | MACS® Annexin V (-) autoclaving | MACS® Annexin V (+) autoclaving |
|---|---|---|---|---|
| Intrinsic diameter, nm | 32 | 31 | 99 | 105 |
| Number average diameter nm | 21 | 19 | 56 | 60 |
| Zeta potential mV | +15 | +19 | -11 | Not determined |
| Fe Concentration | 0.15 | 0.15 | 0.11 | Not determined |
| Solvent | PBS | PBS | HEPES, NaCl, $Ca^{++}$, $Mg^{++}$ | HEPES, NaCl, $Ca^{++}$, $Mg^{++}$ |
| Activity | 100% | 98% | 100% | 43% |
| Bioburden | 0 cfu/2ml | 0 cfu/2ml | 0 cfu/2ml | Not determined |
| Endotoxin | <2.5 | <2.5 | <2.5 | Not determined |

**Table 5:** Physical Properties and Chemical Characteristics. Viahance is the only cell purification agent evaluated that can assure sterility by autoclaving. Annexin-V-based products, like MACS® Annexin V, are unstable to autoclaving as would be expected due to its protein based active agent.