NATE™, a nucleic acid transfection enhancer, has been designed by InvivoGen to specifically increase both transient and stable transfection efficiencies in hard-to-transfect immune cell lines such as THP-1 (Figure 1 and Figure 2) and RAW 264.7 (Figure 3 and Figure 4). NATE™ can be added before all commonly used protocols including various transfection reagents and electroporation. Specifically in RAW 264.7 cells, individual stable clones can be obtained in less than 2 weeks without the need to first establish stable clone pools (Figure 5). Additionally, NATE™ increases the transfection efficiency of larger plasmids (>10 kb) into these hard-to-transfect cell lines. Notably, NATE™ is gentle on cells during the transfection protocol (data not shown).

Greater transient transfection efficiency of THP-1 cells

In the human monocyte cell line, THP-1, the use of NATE™ clearly increases DNA expression when using a GFP-expressing plasmid (Figure 1). Specifically, the transfection efficiency is increased 2-4 fold (green bars) for a small plasmid (~3kb) with both chemical (such as GeneXPlus, Lipofectamine® LTX, and jetPRIME®) and physical (such as nucleofection) transfection protocols (Figure 2, left). Furthermore, this efficiency is enhanced for larger plasmids (~8kb) with a 10x fold increase in transient transfection achieved using the transfection reagent GeneXPlus (Figure 2, right).

Figure 1: Transient transfection of an ~3 kb GFP-expressing plasmid into THP-1 cells was performed using GeneXPlus both in the absence (left) and presence (right) of NATE™. After 48 hours, the transfected cells expressing GFP were visualized by fluorescence microscopy.

Figure 2: Transient transfections of an ~3 kb GFP-expressing plasmid (left) and an 8 kb GFP-expressing plasmid (right) into THP-1 cells was performed using commonly used transfection methods such as GeneXPlus, Lipofectamine® LTX, jetPRIME®, and nucleofection. This was performed both in the presence (green) and absence (yellow) of NATE™. After 48 hours, transfection efficiency (% GFP-expressing cells) was measured using flow cytometry. Data are presented as a fold change compared to transfection without NATE™.
Greater transient transfection efficiency of RAW 264.7 cells

In the murine macrophage cell line, RAW 264.7, the use of NATE™ clearly increases DNA expression when using a GFP-expressing plasmid (Figure 3). Specifically, the transient transfection efficiency is increased 2-5 fold (green bars) for small (~3kb) (Figure 4, left) and larger plasmids (~8kb) (Figure 4, right) with both chemical (such as Lipofectamine® LTX, jetPRIME®, and FuGENE®) and physical (such as nucleofection) transfection protocols.

![Figure 3: Transient transfection of an ~3 kb GFP-expressing plasmid into RAW 264.7 cells was performed using Lipofectamine® LTX both in the absence (left) and presence (right) of NATE™. After 48 hours, the transfected cells expressing GFP were visualized by fluorescence microscopy.](image)

![Figure 4: Transient transfection of an ~3 kb GFP-expressing plasmid and an 8 kb GFP-expressing plasmid into RAW 264.7 cells was performed using Lipofectamine® LTX, jetPRIME®, FuGENE®, and nucleofection. This was performed both in the presence (green) and absence (yellow) of NATE™. After 48 hours, transfection efficiency (% GFP-expressing cells) was measured using flow cytometry. Data are presented as fold change compared to transfection without NATE™.](image)

Increased number of stable SEAP-expressing RAW 264.7 clones

In RAW 264.7 cells, an increased number of stable secreted embryonic alkaline phosphatase (SEAP)-expressing clones is seen with NATE™. These were identified using QUANTI-Blue™ Solution, a SEAP detection reagent, with the purple/blue color indicating stable SEAP expression in the clone present in that well (Figure 5). These stable clones were achieved 10 days after selection, with no need to first establish stable pools before selecting the individual clones.

![Figure 5: Stable transfection of an ~10 kb SEAP-expressing plasmid into RAW 264.7 cells was performed using Lipofectamine® LTX both in the absence (left) and presence (right) of NATE™. After 10 days in selection with Blasticidin, the number of stable clones expressing SEAP (blue wells) was visualized using QUANTI-Blue™, a SEAP detection reagent.](image)