Visartha Agri Sciences

Accelerate your R&D

VisTrans Plant Transfection Reagent

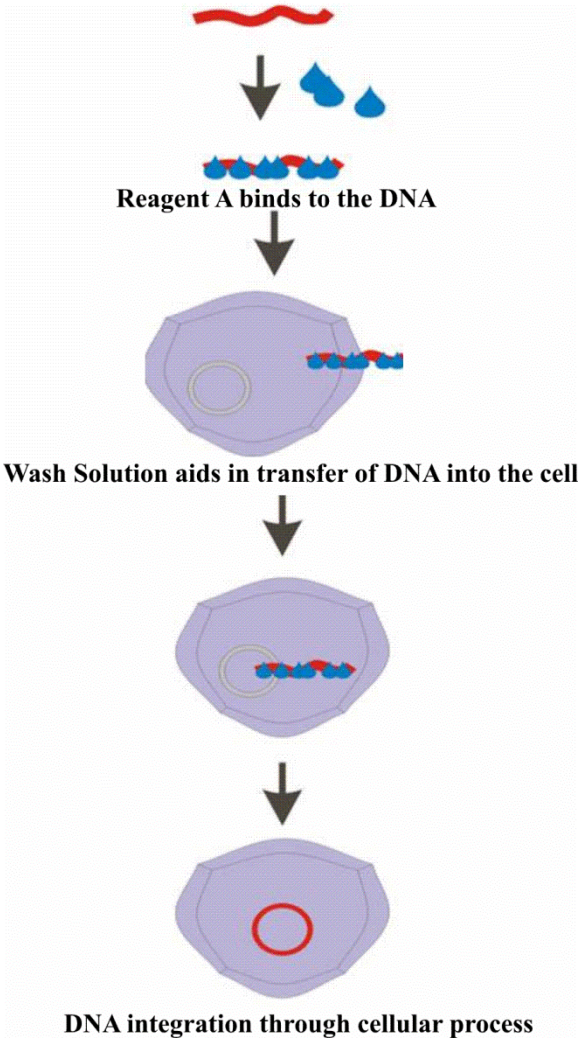
Product Manual V1.0

Cat No: VAS20191
VAS20192

Introduction:

Vistrans Plant transfection kit was developed for direct transformation of plants using linear double stranded DNA and designed to achieve greater transformation efficiencies than the existing methods. The system eliminates the need of using Agrobacterium or specialised instrumentation usage. The reagent can be easily streamlined with existing lab regeneration and transformation protocols.

The system uses a combination of reagents for efficient DNA delivery directly into the plant tissues. The wash buffer tested across species and tissue types transiently permeabilises the cell walls/membranes which are the primary barrier for direct delivery of DNA into the plant tissues. When the DNA to be transfected is added to Reagent A it forms a complex that protects and aids in delivery into the cells.



Protocol:

DNA Preparation

- The system requires a linear double stranded DNA for optimum transfection efficiencies.
- Prepare your DNA by restriction digestion and gel eluting the required fragment that is to be transfected or by amplifying the DNA of your interest using high fidelity DNA Polymerases.
- The typical DNA required amounts are 1 microgram at a concentrations of greater than 50ng/µl

Explant Preparation

- For invitro cultures involving tissue culture prepare your explants according to your lab protocols.
- Collect the explants in appropriate container (sterile petri plates or glass bottles) in sterile double distle/millique grade water that is easy to wash the explants.
- Perform two washes with sterile water to remove any carried out gelling agents and suspend in either 25 ml or 50 ml of sterile water to freely suspend the explants.

DNA Complex Preparation

The concentration of DNA required for optimum transfection efficiencies vary with the size of DNA. Typical concentrations required for DNA with sizes ranges are for <5 Kb use 500ng, for 5-10 Kb use 1µg and for >10 kb use 1.5-2µg of DNA.

Component	<5kb	5-10kb	>10kb
DNA	500 ng	1µg	1.5-2 µg
Reagent A	3 µl	3 µl	3 µl
Nuclease free water	X µl	X µl	X µl
Total Volume	25 µl	25 µl	25 µl

- Prepare the DNA-Reagent A complex by adding the required components, mix them properly with a pipette and let stand the complex for at least 15 minutes at room temperature.
- **IMPORTANT**: The prepared complex has to be added to the explants only after 15 minutes of DNA addition to Reagent A and **within 15 minutes of complex formation**.

Pre-Wash of explants

- Once the explants are ready and resuspended in 25 ml of sterile water, add 0.5ml of wash solution to it and mix immediately by gentle swirling of the container. Mix the contents intermittently for 10-15 minutes.
- Ensure that the explants are not overcrowded and could move freely.
- Ensure that all the explants are wet. Some explants with waxy layers are difficult to wet and in such cases gently submerge the explants using a forceps.
- Number or quantity of explants depends on the volume of wash solution and could be scaled up along with wash buffer/DNA complex volumes.

Treatment with DNA complex

- Discard the wash solution and add fresh 25 ml of sterile water to the explants.
- Add the prepared DNA complex to the explants evenly. This can be achieved by adding few drops at different locations of the container and immediately mixing the contents. Incubate for 15-20 minutes. Swirl the contents gently as many times as possible.
- After the incubation time wash the treated explants with sterile water for at least 3 times. Transfer the explants for to a sterile filter paper for removing the traces of water and drying.
- Transfer the explants to the appropriate regeneration medium.

Post Transfection

- Typical expression of the transfected DNA starts from 36-48 hrs post treatment and for transient expression studies the explants can be observed after 48 hrs.
- For stable transformation sub culture the explants to selection medium only after 2 days or more according to your protocol.
- Observe the explants when using for any excessive damage from the treatment such as increased transparency of leafs, dark brown spots etc., In such cases, decrease the amount of time in wash buffer. It is highly recommended to test the regeneration efficiency of the treated explants.

Disclaimer: Although high care, several optimization studies, testing across species has been carried out the results can widely vary with experiments, explants, varieties and plant species. The liability includes only replacing the kit or its value.

Kit Components & Storage

The kit contains enough components for 10 transformations with explants in a volume of 50ml.

Catalagoue number	50X Wash Solution	Reagent A
VAS20191	2ml X 5	30ul
VAS20192	2ml X 10	60 ul

- Store wash solution at room temperature (20-28^{0}C) and Reagent A at 4^{0}C. Open the contents in a sterile environment to avoid contamination. **Handle Reagent A on ice during complex preparations**.
- The kit is stable upto 1 year from the date of manufacture under the recommended conditions.

Odering Information

Cat. No	Description	Size
VAS20191	VisTransPlant Transfection Reagent	10 Reactions
VAS20192	VisTransPlant Transfection Reagent	20 Reactions

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