Calculation of optimum number of enzyme units

$$\frac{\text{length of your DNA [bp]}}{\text{number of restriction sites}} = \text{your site frequency [bp]}$$

$$\frac{48502\ \text{bp}}{\text{number of } \lambda \text{ restriction sites}} = \lambda \text{ site frequency [bp]}$$

$$\frac{\lambda \text{ site frequency}}{\text{your site frequency}} = \text{number of units for 1 µg DNA} = X_1$$

$$X_1 \times \text{mass of DNA [µg]} = \text{number of units needed for digestion}$$

If the enzyme doesn't cut λ DNA, often Adenovirus 2 DNA is used, which is 35937 bp long.

Example:
5 µg of a 5000 bp plasmid carrying 5 enzyme sites shall be digested.
→ your site frequency = 5000 / 5 = 1000
The same enzyme cuts phage λ DNA also 5 times.
→ λ site frequency = 48502 / 5 = 9700 ≈ 10000
→ number of units for digestion of 1 µg DNA = 10000 / 1000 = 10
→ number of units for digestion of 5 µg DNA = 10 x 5 = 50

- Supercoiled plasmids need more unit activity (up to 5x more) for complete cleavage compared to linearized DNA.
- Use a maximum of 10% enzyme in the total reaction volume, otherwise the glycerol will inhibit the reaction.
- All of Roche's enzymes have a concentration of 10 units/µl

Length of your DNA [bp]	Sites in your DNA	Sites in λ DNA
	1	
Amount of DNA [µg]	Units needed	
1	#DIV/0!	

Length of your DNA [bp]	Sites in your DNA	Sites in Ad2 DNA
	1	
Amount of DNA [µg]	Units needed	
1	#DIV/0!	

https://www.roche-applied-science.com/sis/cloning/cloning.jsp?id=010103

Examples of enzymes assayed with λ DNA:
ApaI: 1 site (prev. dig. with HindIII (6 sites))
EcoRV: 21 sites
HpaI: 14 sites
KpnI: 2 sites
MunI: 8 sites
NdeI: 7 sites
NheI: 1 site (prev. dig. with EcoRI (5 sites))
ScaI: 5 sites
StuI: 6 sites
XhoI: 1 site

Examples of enzymes assayed with Ad2 DNA:
NotI: 7 sites
SpeI: 3 sites