

Gram Stain Protocol

Table 1. Gram stain modifications, recommended reagents, timing and uses^a

Stain and use	Hucker's		Carbol-fuchsin		Kopeloff's	
	Reagent	Time	Reagent	Time	Reagent	Time
Initial stain	Crystal violet	30 s	Crystal violet	30 s	Alkaline crystal violet; flood with solution A; add 5 drops of solution B	2-3 min
Iodine	Gram's iodine	30 s	Gram's iodine	30 s	Kopeloff's iodine	= 2 min
Decolorizer	Acetone-alcohol	~1-5 s	95% ethanol and reagent grade acetone	~30 s	3:7 acetone-alcohol	Rinse immediately after applying
Counterstain	Safranin ^b	30 s	Carbol-fuchsin or 0.8% basic fuchsin	= 1 min	Kopeloff's safranin	10 – 30 s
Recommended use	General bacteriology		<i>Bacteroides</i> spp. <i>Fusobacterium</i> spp. <i>Legionella</i> spp. <i>Campylobacter</i> spp. <i>Brucella</i> spp. and other faintly staining gram-negative organisms		Anaerobes	

^aAdapted from EPCM (see Supplemented Reading)

^bSome laboratories may prefer to use 0.1 to 0.2% basic fuchsin as a counterstain.